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# A spectroscopic investigation into the reaction of sodium tetrathionate with cysteine

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**Abstract**

A spectroscopic investigation into the reaction of sodium tetrathionate with cysteine at pH 5 both at the boil and at room temperature has been carried out. The Raman and infrared spectra of the model compounds cysteine, cysteine-*S*-sulfonate, cysteine-*S*-thiosulfonate, sodium thiosulfate and sodium sulfite were also obtained and vibrations involving the sulfur atoms were analyzed in detail. These results were utilized in the interpretation of the spectra obtained from tetrathionate–cysteine reaction mixtures. The reaction supernatants were analyzed by high performance thin layer chromatography while the precipitates were analyzed gravimetrically. It was found that during the reaction, the thiol groups of cysteine are oxidised to give predominantly cysteine-*S*-sulfonate. Cystine was also detected but was determined gravimetrically to be a minor reaction product. No significant amounts of cysteine-*S*-thiosulfonate were detected. The reaction is accompanied by the formation of elemental sulfur and a small amount of sulfite. Major reaction pathways are put forth that are consistent with the experimental data.


*Keywords:* Tetrathionate; Cysteine; Cysteine-*S*-sulfonate; Cysteine-*S*-thiosulfonate; Cystine; Infrared spectroscopy; Raman spectroscopy

## 1. Introduction

The reaction of thiols with sodium tetrathionate in aqueous alkaline solution to give disulfides is believed to involve two nucleophilic displacements as shown in reactions (1) and (2) [1]:

$$RS^- + {}^-O_3S\text{–}S\text{–}S\text{–}SO_3{}^- \rightleftharpoons RS\text{–}S\text{–}SO_3{}^- + S_2O_3{}^{2-} \quad (1)$$

$$RS\text{–}S\text{–}SO_3{}^- + RS^- \rightleftharpoons RS\text{–}SR + S_2O_3{}^{2-} \quad (2)$$

Nucleophilic attack by the thioate anion on the inner sulfur of tetrathionate gives an alkyl-*S*-thiosulfonate as an intermediate which then undergoes further nucleophilic attack to give the disulfide plus thiosulfate. This mechanism has been proposed for the blocking of thiol groups by sodium tetrathionate in proteins [2,3] and in wool where the blocking of thiol groups inhibits thiol-disulfide interchange during dyeing [4].

The oxidation of cysteine by sodium tetrathionate in aqueous solution however appears to be more complicated than depicted by the above reactions and Szczepkowski [5] observed the formation of cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate in addition to cystine in the reaction mixture. More recently Inglis and Liu [6] used the reaction of cysteine with sodium tetrathionate in unbuffered aqueous solution to prepare cysteine-*S*-sulfonate (cysteine Bunte salt).

The formation of cysteine-*S*-sulfonate in addition to cysteine-*S*-thiosulfonate (reaction (1)) may be explained by nucleophilic attack by cysteine thiolate anion on the outer sulfur of tetrathionate as shown in reaction (3):

$$RS^- + {}^-O_3S\text{–}S\text{–}S\text{–}SO_3{}^- \rightleftharpoons RS\text{–}SO_3{}^- + {}^-S\text{–}S\text{–}SO_3{}^- \quad (3)$$

This mechanism was first proposed by Stelmaszynska and Szczepkowski [7] to explain the multiple products formed in the reaction of cysteine with sodium pentathionate ($Na_2S_5O_6$). Dithiosulfate anions (${}^-S\text{–}S\text{–}SO_3{}^-$) are proposed to be formed in this reaction. In acid solution these ions decompose to give elemental sulfur and sulfite in an analogous manner to the acidic breakdown of thiosulfate according to the overall equation shown by reaction (4) [8,9]:

$$S_2O_3{}^{2-} + H^+ \rightleftharpoons HSO_3{}^- + S \quad (4)$$

The reaction shown above as Eq. (4) is actually a complex cascade of reactions building up to ${}^-S\text{–}(S)_7\text{–}SO_3{}^-$ which then gives

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elemental sulfur and sulfite. On the basis of this discussion, for the reaction of sodium tetrathionate with cysteine we could expect to see the formation of sulfur from the decomposition of dithiosulfate as well as cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate. In an earlier study of the reaction of cysteine with sodium tetrathionate the formation of elemental sulfur was not reported [5].

Recently we reported on the products of reaction of cysteine with sodium tetrathionate in aqueous solution over the pH range 3–7 [10]. High performance thin layer chromatography (HPTLC) results obtained suggested that cysteine-*S*-sulfonate was the major product. Raman spectroscopy provided evidence that elemental sulfur was also produced. These observations suggest that reaction (3) is the predominant reaction with the forward direction of reactions (1) and (2) being minor pathways. To build further support for these findings we have carried out a detailed spectroscopic investigation of the reaction mixtures at pH 5 both at the boil and at room temperature. Spectroscopic analysis of model compounds has been used to aid in the spectral interpretation.

## 2. Experimental

### 2.1. Materials

Sodium tetrathionate and cystine were purchased from BDH chemicals. Cysteine and cysteine-*S*-sulfonate (*S*-sulfocysteine) were purchased from Fluka. Cysteine-*S*-thiosulfonate[1] was synthesised by reaction of *S*-(2-amino-2-carboxyethylsulfonyl)-L-cysteine with thiosulfate as described by Ubuka et al. [11]. The *S*-(2-amino-2-carboxyethylsulfonyl)-L-cysteine was prepared using the alternative method of Toennies and Lavine and substituting *m*-chloroperbenzoic acid (Fluka) for the perbenzoic acid [12]. The purity of the model compounds were checked by HPTLC.

Analytical grade sodium thiosulfate pentahydrate and sodium sulfite were purchased from Ajax and Aldrich, respectively. The McIlvaine buffer [13] was prepared from disodium hydrogen phosphate and citric acid, both of which were provided by BDH chemicals.

### 2.2. Reaction of cysteine with sodium tetrathionate

Cysteine (0.1 mmol in 0.1 M McIlvaine buffer, pH 5) and sodium tetrathionate (0.1 mmol in the same buffer) were mixed in a test tube. A total reaction volume of 10 mL was used. The reaction mixture was heated in a boiling water bath for 1 h. After cooling to room temperature, the tube was centrifuged (5 min at 4000 rpm) and the supernatant was separated from the precipitate.

A sample of the supernatant was taken for HPTLC analysis. The remainder of the supernatant and all of the precipitate was allowed to evaporate to dryness at room temperature and then held in a 40 °C oven for 24 h to drive off any remaining water. The dry weight of the precipitate was determined. A second reaction was carried out by allowing the mixture to sit at room temperature (~23 °C) for 72 h. The reaction products were worked up in a method similar to that given above.

### 2.3. HPTLC

HPTLC was conducted on silica plates (Merck PF 254) using a Camag Nanomat applicator. The plates were run using butanol:acetic acid:water (12:3:5) as the solvent. Spots were identified by examination under UV light, acidic ninhydrin reagent [14] or exposure to iodine vapor.

### 2.4. Infrared and Raman spectroscopy

Infrared spectra were recorded at a resolution of 4 $cm^{-1}$ using a Perkin-Elmer System 2000 FT-IR spectrometer. All samples were studied using the KBr pellet technique. Raman spectra were recorded at 4 $cm^{-1}$ resolution using a Bruker RFS-100 FT-Raman spectrometer fitted with an Aldas Nd:YAG laser operating at 1064 nm. Samples were held in standard 2 mm cavity cells. The Raman spectrum of elemental sulfur was obtained using the Bruker calibration standard.

## 3. Results and discussion

### 3.1. IR and Raman spectroscopy of model compounds

The main reaction products of interest in this study are cystine (R–S–S–R), cysteine-*S*-thiosulfonate (R–S–S–$SO_3$) and cysteine-*S*-sulfonate (R–S–$SO_3$). All of the fundamental vibrational modes associated with the sulfur atoms are expected to be observed below 1450 $cm^{-1}$. The low wavenumber Raman and infrared spectra obtained from these compounds are shown in Figs. 1 and 2, respectively. While the fundamental vibrations of the C–S–S–C linkage are skeletal, those of the C–$(S)_n$–$SO_3$ moiety can be broken up into three groups, the $SO_3$ stretching vibrations, the $SO_3$ deformations and the skeletal vibrations. Considering the lack of symmetry in these molecules, all of these vibrations would be expected to be both infrared and Raman active.

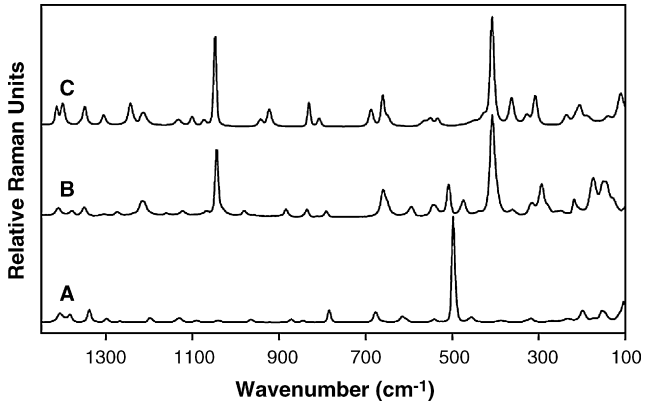


Fig. 1. Raman spectra (1450–100 $cm^{-1}$) of (A) cystine, (B) cysteine-*S*-thiosulfonate and (C) cysteine-*S*-sulfonate.

[1] Systematic name: 2-amino-2-carboxyethyl sulfodisulfane.

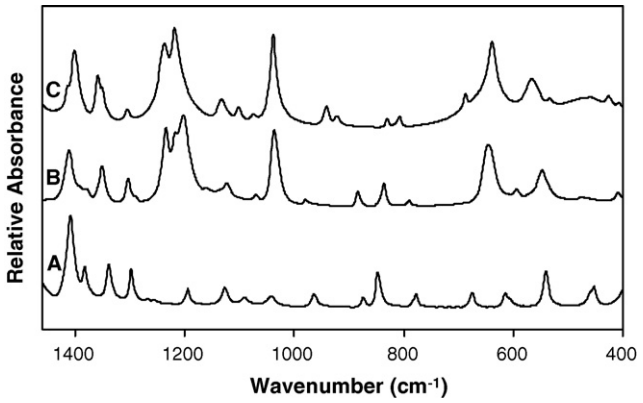


Fig. 2. Infrared spectra (1450–400 $cm^{-1}$) of (A) cystine, (B) cysteine-*S*-thiosulfonate and (C) cysteine-*S*-sulfonate.

The assignments of the $SO_3$ stretching modes are fairly straightforward [15–18]. The anti-symmetric modes for the cysteine-*S*-thiosulfonate and cysteine-*S*-sulfonate groups are assigned to moderate Raman lines observed at 1215 and 1214 $cm^{-1}$, respectively. In the infrared this mode is very strong and split into several components. For the cysteine-*S*-sulfonate, these components are observed at 1237 and 1218 $cm^{-1}$ while for the cysteine-*S*-thiosulfonate they appear at 1234, 1216 and 1202 $cm^{-1}$. The additional component at 1202 $cm^{-1}$ is not consistent with what is observed for the cysteine-*S*-sulfonate and none of the other degenerate modes exhibit more than two components.

The symmetric $SO_3$ stretching modes of the cysteine-*S*-thiosulfonate and cysteine-*S*-sulfonate groups are assigned to very strong Raman lines observed at 1044 and 1048 $cm^{-1}$, respectively. In the infrared these modes have been assigned to the very strong sharp symmetric features observed at 1038 and 1032 $cm^{-1}$. From these assignments it is clear that the frequencies of the $SO_3$ stretching modes of cysteine-*S*-thiosulfonate and cysteine-*S*-sulfonate are very similar. In actual protein molecules, where more conformational and environmental diversity is expected, the $SO_3$ stretching vibrations would be broadened thus limiting the ability to distinguish between the two species.

The $SO_3$ deformation modes can also be confidently assigned based on the literature [16–18]. The symmetric deformations of cysteine-*S*-thiosulfonate and cysteine-*S*-sulfonate can be assigned to the moderately intense infrared bands observed at 645 and 639 $cm^{-1}$, respectively. These modes appear as weak shoulders at 650 $cm^{-1}$ in the Raman. The anti-symmetric $SO_3$ deformations of cysteine-*S*-thiosulfonate are assigned to strong infrared band at 547 $cm^{-1}$ and the weak Raman line at 544 $cm^{-1}$. The corresponding vibrations of cysteine-*S*-sulfonate are both observed at 567 $cm^{-1}$.

Due to the possibility of rotational conformers, the assignments of the skeletal modes are expected to be quite complex. Of these modes, the C–S and S–S stretching vibrations are expected to be the most useful in terms of qualitative analysis. The assignments of the C–S [15,16,19] and S–S [15,17–19] stretching vibrational modes of cysteine are well established. The C–S stretching mode is assigned to the moderately intense Raman line observed at 676 $cm^{-1}$. The corresponding infrared mode is observed at 675 $cm^{-1}$. The S–S stretching vibration is assigned to the strongest feature in the Raman spectrum observed near 500 $cm^{-1}$. As expected, this mode is observed as a weak feature at 499 $cm^{-1}$ in the infrared.

The C–S stretching vibrations of both cysteine-*S*-thiosulfonate and cysteine-*S*-sulfonate are observed at 660 $cm^{-1}$. They appear as weak shoulders in the infrared and as medium intense lines in the Raman. While both cysteine-*S*-thiosulfonate and cysteine-*S*-sulfonate exhibit strong S–$SO_3$ lines near 408 $cm^{-1}$, the *S*-thiosulfonate also exhibits a moderate line at 509 $cm^{-1}$. This latter line has been tentatively assigned to the S–S stretching vibration in accordance with the corresponding vibration observed in the spectrum of cystine. It is interesting to note that Lecomte et al., in their study of polythionates [17], did not observe the S–S stretching vibration (495 and 504 $cm^{-1}$) until the chain length reached five sulfur atoms.

The S–S–O deformations of the cysteine-*S*-thiosulfonate are assigned to the weak and moderate Raman lines observed at 327 and 309 $cm^{-1}$. The corresponding vibrations of the *S*-sulfonate are observed at 314 and 292 $cm^{-1}$. These assignments are consistent with those for the corresponding vibrations of similar molecules presented in the literature [16,17]. The observed frequencies and assignments presented above for cysteine-*S*-sulfonate, cysteine-*S*-thiosulfonate and cystine are summarized in Table 1. In comparing the spectra obtained from the model compounds, the most distinctive differences are in the Raman lines associated with the S–S and S–$SO_3$ stretching modes. From this result it is clear that the frequencies of the disulfide stretching vibrations can be used to distinguish between cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate groups present in the reaction mixture.

According to Eqs. (1)–(3), sodium sulfite and sodium thiosulfate can be present in the reaction mixtures. The fundamental vibrations of these ions are very similar to those expected for the corresponding functional groups in cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate. The ability to identify these species in the spectra obtained from the reaction mixtures is thus very important. While the vibrational assignments of the sulfite and thiosulfate ions are well established [16,18,20], the observed frequencies have not been presented in detail for the solid phase. In order to obtain accurate solid state frequencies for use in comparison to the spectra obtained from our reaction mixtures, we have investigated the infrared and Raman spectra of sodium sulfite and sodium thiosulfate pentahydrate in the solid state. As free ions, both thiosulfate and sulfite have $C_{3v}$ symmetry and 9 ($3A_1 + 3E$) and 6 ($2A_1 + 2E$) vibrational modes are expected, respectively.

The observed vibrational frequencies and assignments for solid sodium thiosulfate pentahydrate and sodium sulfite are presented in Table 2. The assignments given are consistent with those reported in the literature [18,20]. We have observed however that most of the thiosulfate bands appear to exhibit multiple splitting, four bands instead of two for the E modes and two bands instead of one for the $A_1$ modes. The splitting can be attributed to a reduced site and crystal symmetry ($P21/c$-$C_{2h}^5$)

Table 1
Assignments of the fundamental vibrations incorporating the sulfur atoms of cysteine-*S*-sulfonate (RS–$SO_3$Na), cysteine-*S*-thiosulfonate (RS–S–$SO_3$Na) and cystine (R–S–S–R)

| R–S–$SO_3$Na | | R–S–S–$SO_3$Na | | R–S–S–R | | Assignments |
|---|---|---|---|---|---|---|
| R | IR | R | IR | R | IR | |
| 1214 m | 1237 vs, 1218 vs | 1215 m | 1234 vs, 1216 vs, 1202 vs | – | – | Antisym $SO_3$ Str |
| 1048 vs | 1038 vs | 1044 vs | 1032 vs | – | – | Sym $SO_3$ Str |
| 660 m | 660 w, sh | 660 m | 660 w, sh | 676 m | 675 m | C–S Str |
| 650 w, sh | 639 m | 650 w, sh | 645 m | – | – | Sym $SO_3$ Def |
| 567 w | 567 s | 544 w | 547 s | – | – | Antisym $SO_3$ Def |
| – | – | 509 m | | 500 vs | 499 w | S–S Str |
| 408 vs | 406 w | 407 vs | 407 w | – | – | S–$SO_3$ Str |
| 327 w, 309 m | – | 314 w, 292 m | – | – | – | S–S–O Def |

R = –$CH_2$·$CHNH_2$·COOH, sh = shoulder, s = strong, m = medium, w = weak, v = very.

Table 2
Observed frequencies and assignments for the fundamental vibrations of sodium thiosulfate pentahydrate and sodium sulfite in the solid state

| $Na_2S$–$SO_3$ | | Assignments | $Na_2SO_3$ | | Assignments |
|---|---|---|---|---|---|
| R | IR | | R | IR | |
| 1157 m, 1120 m | 1161 vs, sh; 1146 vs, sh; 1134 vs; 1113 vs | $\nu_4$(E) Antisym $SO_3$ Str | 986 vs | 968 vs | $\nu_1(A_1)$ Sym $SO_3$ Str |
| 1016 m, 991 m | 1000 s | $\nu_1(A_1)$ Sym $SO_3$ Str | 948 m | – | $\nu_3$(E) Antisym $SO_3$ Str |
| 671 m, sh; 656 m | 671 s, 664 s | $\nu_2(A_1)$ S–$SO_3$ Str | 637 w | 631 m | $\nu_2(A_1)$ Sym $SO_3$ Def |
| 550 w, 527 w | 552 m, 527 m | $\nu_5$(E) Antisym $SO_3$ Def | 496 m | 495 m | $\nu_4$(E) Antisym $SO_3$ Def |
| 452 vs, 433 vs | 452 vw, 426 vw | $\nu_3(A_1)$ Sym $SO_3$ Def | – | – | – |
| 360 m, sh; 343 m; 323 m | – | $\nu_6$(E) S–S–O Def | – | – | – |

sh = shoulder, s = strong, m = medium, w = weak, v = very.

and the four molecules per unit cell [21]. The observed frequencies for the sulfite and thiosulfate ions in the solid state are significantly shifted from those of the cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate (see Table 1). The presence of these inorganic species should therefore be easily detected in the reaction mixtures.

### 3.2. Reactions of cysteine with tetrathionate

On boiling equimolar amounts of cysteine and sodium tetrathionate at pH 5 the solution became turbid within a few minutes and exhibited a significant odor of sulfite during heating. After 60 min at the boil a yellowish-white solid precipitated. Upon settling the precipitate was found to form two layers, a white bottom layer and a yellow upper layer. The reaction carried out at room temperature progressed at a much slower rate but similar observations were made.

The reaction supernatants were analyzed by HPTLC. The model compounds cysteine, cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate were run as reference standards. The reaction supernatants showed three components. The major ninhydrin positive spot at $R_f$ 0.14 migrated with the same mobility as cysteine-*S*-sulfonate in agreement with the result of Inglis and Liu [6]. A second, very faint ninhydrin positive sport was also observed at $R_f$ 0.24 with the same mobility as cysteine-*S*-thiosulfonate. This was very close to the migration of cysteine ($R_f$ 0.26) so it was difficult to conclusively identify this component. However, based on previous work on the reaction of *N*-acetylcysteine with sodium tetrathionate under identical conditions [10] it was established that no *N*-acetylcysteine remained after reaction for 1 h at the boil or 48 h at room temperature. On this basis we can be reasonably confident that no cysteine remained in the supernatant at the end of the reaction.

In a recent study of the reaction of tetrathionate with cysteine present in wool [10] it was found that Raman lines from the highly abundant disulfide bonds of cystine interfered with the analysis of lines associated with cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate residues. Unlike the complex situation in wool, the reaction mixtures of the model compounds are self-simplifying. Cystine is not soluble in water at neutral pH and is expected to be in the precipitate [22]. Both cysteine-*S*-sulfonate and cysteine-*S*-thiosulfonate are soluble in water and if present would be expected to be found in the supernatant. The only compounds expected to complicate the spectra obtained from the reaction supernatant are those associated with the McIlvaine buffer, namely citrate and phosphate. Attempts to carry out the reaction of tetrathionate with cysteine either unbuffered or with less complicated buffers such as acetate or phosphate were not successful. Due to lack of buffering capacity, the pH of the reaction solution was found to quickly become acidic.

Examination of the precipitates revealed that two discrete layers were formed, a fine white layer and a coarser yellow layer. Samples from these layers were isolated and spectroscopically analyzed. The spectra obtained from the layers formed from reactions carried out at the boil as well as at room temperature were found to be very similar. The 1500–250 $cm^{-1}$ region of the Raman spectra obtained from the two layers isolated from a typical reaction precipitate are shown as Fig. 3. A majority of the lines observed in the spectrum obtained from the white layer, trace A, can be associated with cystine (Fig. 1). The very

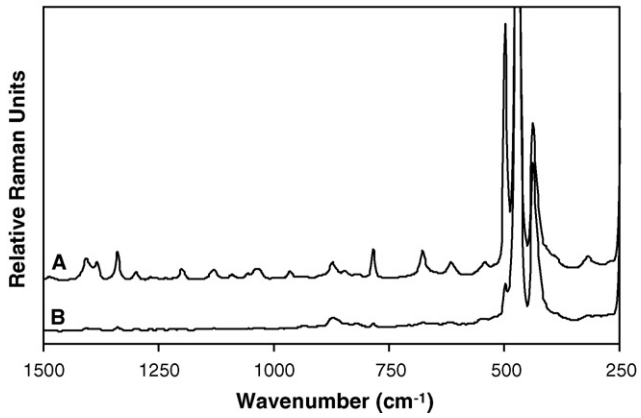


Fig. 3. Raman spectra of the white (A) and yellow (B) layers of the precipitate formed during the room temperature reaction of cysteine with $Na_2S_4O_6$ at pH 5.

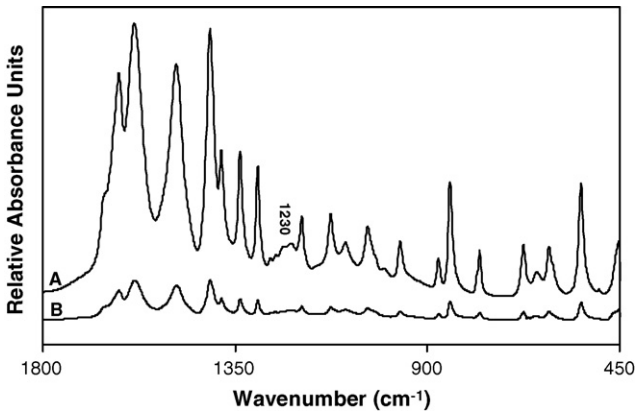


Fig. 4. Infrared spectra of the white (A) and yellow (B) layers of the precipitate formed during the room temperature reaction of cysteine with $Na_2S_4O_6$ at pH 5.

strong and sharp S–S stretching vibration is clearly present at 500 $cm^{-1}$. No evidence of either S–$SO_3$ or $SO_3$ vibrations could be detected suggesting that cysteine-*S*-sulfonate and/or cysteine-*S*-thiosulfonate are not present in the precipitate. The intense lines at 472 and 436 $cm^{-1}$ can be associated with the presence of elemental sulfur. The spectrum obtained from the yellow layer, trace B, reveals that this layer is largely elemental sulfur. Aside from the 472 and 436 $cm^{-1}$ lines, the weak 870 $cm^{-1}$ sulfur line is also observed at this high concentration. The sharp 500 $cm^{-1}$ line as well as the weaker 1398, 1338, 783 $cm^{-1}$ lines confirms the presence of a small amount of cystine in this layer. The cross-contamination of the two layers is not unexpected.

A theoretical yield for cystine can be calculated from the dry weigh of the precipitate based on the combination of the reactions given by Eqs. (1) and (2):

$$2RS^- + {}^-O_3S\text{–}S\text{–}S\text{–}SO_3{}^- \rightleftharpoons RS\text{–}SR + S_2O_3{}^{2-}$$

If it is assumed that cystine is the only product present in the precipitate a yield of 30% is obtained. It is more likely that cystine makes up less than half of the weight of the precipitate making the yield more of the order of 15%. This result suggests that cystine must only be a minor product from the reaction of sodium tetrathionate and cysteine.

The 1800–450 $cm^{-1}$ region of the infrared spectra obtained from these two layers are shown as Fig. 4. Comparable masses of each layer were used to obtain each of the spectra. From comparison of the relative scales of these spectra it is apparent that the major component of the yellow layer is inactive in the infrared. This material is most likely sulfur. If the scale difference is neglected, the spectra obtained from the two layers are actually very similar and consistent with that of the model compound cystine shown in Fig. 2. The only absorbance bands not accountable for by cystine are the very weak features observed near 1230 $cm^{-1}$. This is the general region where the anti-symmetric $SO_3$ stretching modes would be active. From Table 2 it is apparent that the fundamental vibrations of thiosulfate and sulfite are much lower in frequency. The actual frequencies observed, 1218 and 1237 $cm^{-1}$, are in good agreement however with those presented in Table 1 for cysteine-*S*-sulfonate. While the very strong symmetric $SO_3$ stretching mode of cysteine-*S*-sulfonate (1038 $cm^{-1}$) would overlap with a moderately intense cystine band, the strong anti-symmetric $SO_3$ deformational mode should be detectable. This mode can be assigned to a weak shoulder observed at 567 $cm^{-1}$ in the spectrum obtained from the white layer of the precipitate. It is therefore likely that there is a small amount of cysteine-*S*-sulfonate present in the precipitate.

The supernatants isolated from the reaction mixtures were dried to white powders. As there is a 10-fold excess of buffer species compared to reactants, the buffer is expected to be the dominant component. The supernatant residue spectra obtained from the reactions carried out at the boil and at room temperature exhibited some differences and are thus both shown in Fig. 5. The spectrum of the residue obtained after evaporation of an aliquot of the McIlvane buffer is also shown. Unlike the sharp well defined features observed in the spectrum obtained from the white layers of the precipitate formed during the reactions (Fig. 3A), the spectra obtained from the supernatant residues largely exhibited weak broad features similar to those of the McIlvane buffer (Fig. 5C). The strong presence of the buffer in the reaction mixture supernatant is confirmed by observation of

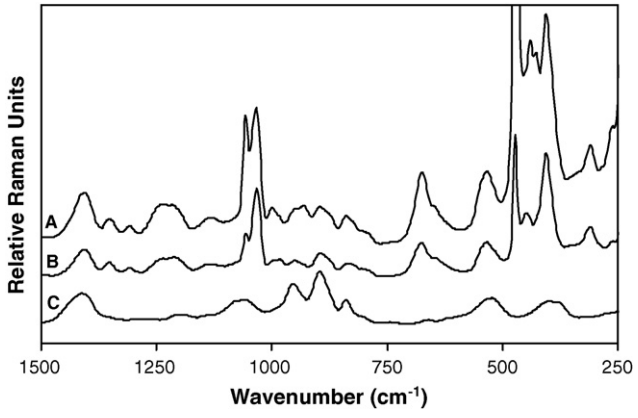


Fig. 5. Raman spectra of the white powder isolated from the supernatant from the reaction of cysteine with $Na_2S_4O_6$ at pH 5: (A) at room temperature, (B) at the boil and (C) spectrum obtained from the residue obtained after evaporation of an aliquot of McIlvane buffer.

intense C–H stretching vibrations at 2936 $cm^{-1}$ (not shown) that can be attributed to citric acid.

The very strong sharp features observed in the spectrum obtained from the supernatant at 472 and 437 $cm^{-1}$ can be attributed to elemental sulfur. A significantly larger amount of sulfur appears to be present in the supernatant of the room temperature reaction mixture. It is quite possible however that this difference is due to the sampling of a heterogeneous reaction mixture residue. Strong features at 1056 and 1032 $cm^{-1}$ can most likely be associated with the S–O stretching vibrations of $SO_3$ groups. Differences can be noted in the relative intensities of the two components making up this line for the two reaction conditions. Additional intense Raman lines are observed at 676 and 405 $cm^{-1}$. The S–H stretching vibration of cysteine is observed as a very strong sharp feature at 2551 $cm^{-1}$. No features were observed in this region of the spectra obtained from the reaction mixtures suggesting that the reaction has gone to completion. This finding is supported by the HPTLC results presented above.

In an attempt to enhance the features due to the reaction products in the supernatant, the spectrum obtained from the buffer residue was subtracted. The result is shown in Fig. 6 along with the spectra obtained from cysteine-*S*-sulfonate (B) and sulfur (C). In this scale expanded plot the strong sulfur lines can clearly be identified at 473, 219 and 1543 $cm^{-1}$. The symmetric $SO_3$ and S–$SO_3$ stretching vibrations present at 1032 and 405 $cm^{-1}$, respectively suggest that either cysteine-*S*-sulfonate and/or cysteine-*S*-thiosulfonate are present in the reaction supernatant mixture. From careful comparisons there is reasonable correspondence in both frequency and relative intensity of many of the broad features observed in the reaction mixture spectra and the sharp features of cysteine-*S*-sulfonate spectra. There is no correlation with the Raman lines of cysteine-*S*-thiosulfonate and in particular, the strong S–S stretching vibration expected at 500 $cm^{-1}$ is clearly not observed.

The chemical composition of the reaction supernatant was further investigated through its infrared spectrum which is shown as trace A in Fig. 7. Unlike the Raman spectrum obtained from the reaction mixture supernatant, the infrared spectrum obtained

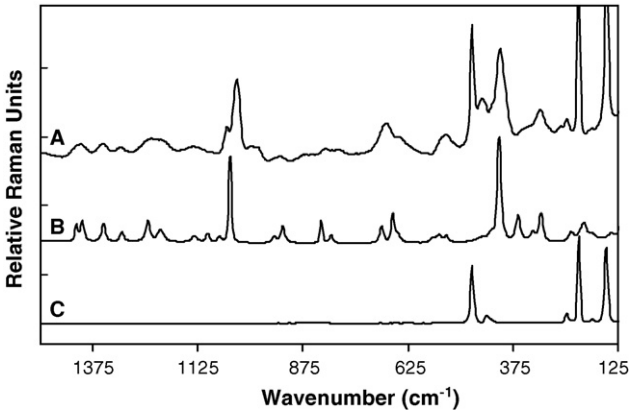


Fig. 6. Raman spectra of (A) the white powder isolated from the supernatant from the reaction of cysteine with $Na_2S_4O_6$ at pH 5: (B) cysteine-*S*-sulfonate and (C) sulfur.

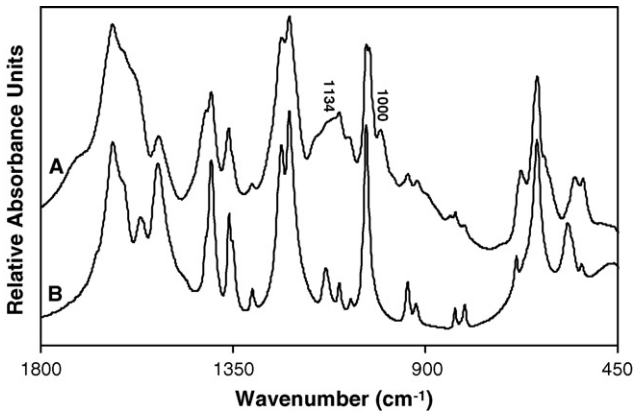


Fig. 7. Infrared spectra obtained from (A) the white powder isolated from the supernatant isolated from the boiled pH 5 reaction mixture of cysteine and $Na_2S_4O_6$ and (B) cysteine-*S*-sulfonate.

exhibits many strong sharp features. From comparison, these features are in excellent agreement with those of cysteine-*S*-sulfonate (trace B). No features can be attributed to the presence of cysteine-*S*-thiosulfonate. The features observed at 1134 and 1000 $cm^{-1}$ are consistent with the presence of inorganic thiosulfate in the mixture.

## 4. Conclusions

The reaction of sodium tetrathionate with cysteine is quite complex. The experimental results presented above for the reaction at pH 5 support the proposal that the major pathway is as follows:

$$RS^- + {}^-O_3S\text{–}S\text{–}S\text{–}SO_3{}^- \rightleftharpoons RS\text{–}SO_3{}^- + {}^-S\text{–}S\text{–}SO_3{}^-$$

Spectra obtained from the reaction mixtures enabled identification of cysteine-*S*-sulfonate as the major product independent of the reaction temperature. No evidence of the cysteine starting material was detected suggesting the reaction has gone to completion. While the amount of cysteine-*S*-thiosulfonate produced appears to be below the detection limits of the spectroscopic techniques, HPTLC results suggested that a small amount was produced. Cystine was also identified as a minor product. These latter results suggest that there are other minor pathways including the forward directions of reactions (1) and (2) given above.

Raman spectroscopy was used to confirm the formation of elemental sulfur as a significant reaction product. A faint odor of sulfite was detected during the reaction but none was detected spectroscopically. The formation of these species can be explained by the overall reaction:

$$S_2O_3{}^{2-} + H^+ \rightleftharpoons HSO_3{}^- + S$$

Fitting in with the above reaction, a minor amount of inorganic thiosulfate was also detected in the infrared spectra obtained from the supernatants of the reaction mixtures. It is likely that that this was formed from the decomposition of the dithiosulfate produced in reaction (3) and the forward direction of reactions (1) and (2).

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