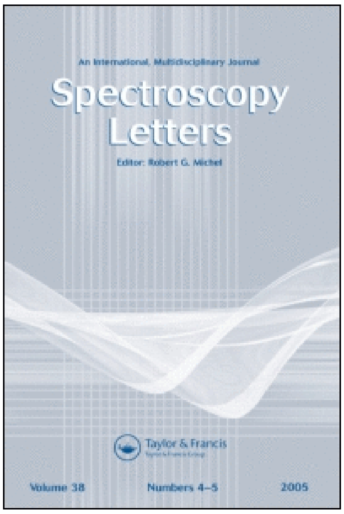

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IR Study on Aqueous Solution Behavior of *D*-Cycloserine

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IR STUDY ON AQUEOUS SOLUTION BEHAVIOR OF *D*-CYCLOSERINE

Key Words : D-Cycloserine, hydrolysis, dimerization,
IR spectra

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ABSTRACT

IR spectra were measured for an antitubercular agent, *D*-cycloserine (CS), and the hydrolyzate, β-

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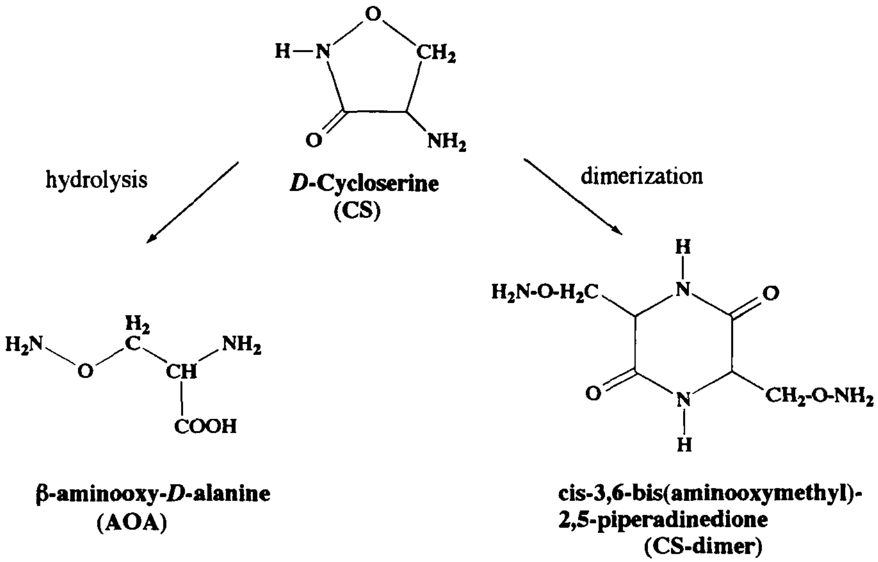
aminooxy-D-alanine (AOA) and the dimer, cis-3,6-bis(aminooxymethyl)-2,5-piperadinedione (CS-dimer) at various pHs in aqueous solutions. Molecular species existing in the ionic equilibria were characterized by the pH dependence of the spectra. Band assignments were carried out by reference to the spectra of D_2O solutions and those of related compounds. Spontaneous transformation of CS to CS-dimer was observed by the IR spectra for the neutral aqueous solution. The spectral evidence suggested that the non-ionic form of CS plays a key role in the dimerization process.

INTRODUCTION

D-4-Amino-3-isoxazolidinone, better known as *D*-Cycloserine (CS) is a broad-spectrum antibiotic produced by the *Streptomyces* family, which is used clinically as an antitubercular agent. Isolation, characterization and chemical synthesis of this antibiotic substance have appeared in a series of publications[1]. It has been also reported that CS undergoes the hydrolysis and the dimerization reactions in aqueous solution[2] (Scheme 1). Since the hydrolyzate, β-aminooxy-D-alanine (AOA) and the dimer, cis-3,6-bis(aminooxymethyl)-2,5-piperadinedione (CS-dimer) are supposed to have biological activities different from CS[3], it is clinically important to understand the aqueous solution properties of CS.

As to the molecular structure of CS, Turley and Pepinsky reported the X-ray crystal structure of CS hydrochloride[4]. Preliminary IR data were also presented for CS, the hydrolyzate and the dimer in the solid state[1]. However, no systematic studies have been carried out so far on the structures and the spectra of



Scheme 1 Hydrolysis and dimerization of CS.
The molecular structures are depicted in their non-ionic forms.

CS and its derivatives in aqueous solution. In this work the IR spectra of CS, AOA and CS-dimer have been measured at a series of pHs. Molecular species at various dissociation states are characterized by the pH-dependent spectra. Further, transformation of CS to CS-dimer is also discussed.

EXPERIMENTAL

Materials

CS was a generous gift from Meiji Seika, Ltd. Japan and recrystallized from 2-propanol/water. AOA was obtained by hydrolysis of CS using 6 M hydrochloric acid and recrystallized from ethanol/water[5]. CS-dimer was synthesized by refluxing CS with glacial acetic acid in absolute ethanol and recrystallized from ethanol/water[2]. 4-Acetamido-3-isoxazolidone (Ac-CS) was prepared by the reaction of CS with acetic anhydride in dichloromethane and recrystallized from ethanol/petroleum ether[6]. The chemical purities of the samples were guaranteed by elemental analysis, and mass and NMR spectra.

Measurement

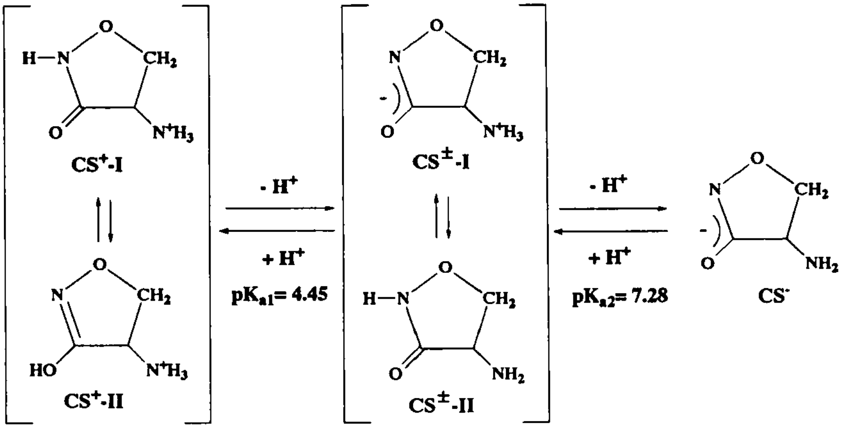
IR spectra were recorded on a Perkin Elmer 1650 FT-IR spectrometer by averaging 64 scans with a resolution of 4 cm^{-1}. The spectra of solid samples were measured as KBr pellets. A horizontal attenuated total reflection cell with a ZnSe crystal was used for the measurement of the aqueous solutions. The pH values of the samples were adjusted using 1 M NaOH and 1 M HCl solutions. The same concentrations of NaOD and DCl solutions were used for D_2O solutions.

RESULTS AND DISCUSSION

pH Dependence of IR Spectra in Aqueous Solution

CS

In view of the two ionizable groups, the ionic and tautomeric equilibria of CS can be illustrated as in Scheme 2. The pK_{a1} and pK_{a2} values were ascribed to the dissociation of the enolic hydroxyl and the ammonium groups, respectively[7]. In order to identify the molecular species in each dissociation state, IR spectra



Scheme 2 Dissociation and tautomerism of CS in aqueous solution.

of CS were measured in H_2O (D_2O) solutions at pHs (pDs) below and above the pKa values. Figs. 1 and 2 show IR spectra of CS in H_2O and D_2O solutions. Table 1 summarizes IR frequencies and band assignments. The spectra were acquired just after the sample preparation in order to avoid the effect of the dimer formation as discussed later. Spectral patterns change reversibly according to the pH changes. At pH 3.6, the IR spectrum shows an intense band at 1715 cm^{-1}, weak bands at 1613 and 1573 cm^{-1} and a medium peak at 1538 cm^{-1}. In D_2O solution the 1715 cm^{-1} band shifts to 1698 cm^{-1}, and the 1613 and 1538 cm^{-1} bands are not observed. The latter two bands are assignable to the NH_3^+ deformation modes. The observation of the C=O stretching band at 1715 cm^{-1} indicates that CS exists as a mono cationic species, CS^+-I, and not as the CS^+-II form, in acidic solution. CS hydrochloride was reported to have the CS^+-I form in the crystalline state[4]. The IR spectrum of this crystal

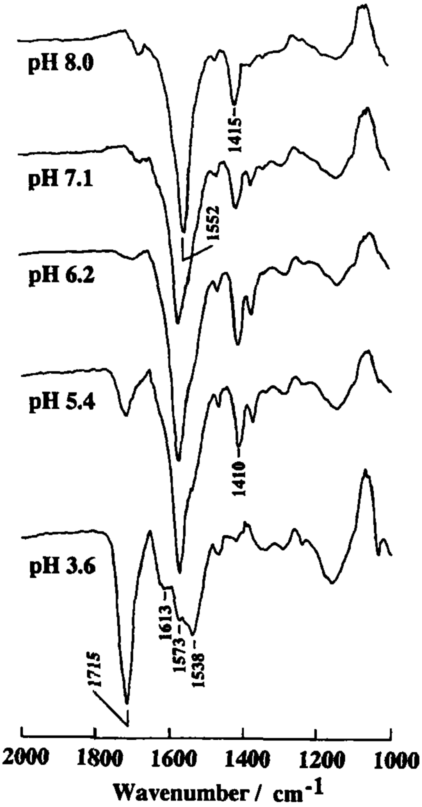


FIG. 1 IR spectra of CS in H_2O solution.

shows the C=O stretching and the NH_3^+ deformation mode at 1701 and 1618 cm^{-1}, respectively, comparable to those in the pH 3.6 solution. As the pH is raised above the pK_{a1} value, the 1573 cm^{-1} band increases its intensity at the expense of the 1715 cm^{-1} band. At pH 6.2 the latter is completely replaced by the former, and a new weak band is observed at 1410 cm^{-1}. This 1573 cm^{-1} band is observed at a higher frequency by 9 cm^{-1} in D_2O solution, whereas the 1410 cm^{-1} band does not shift.

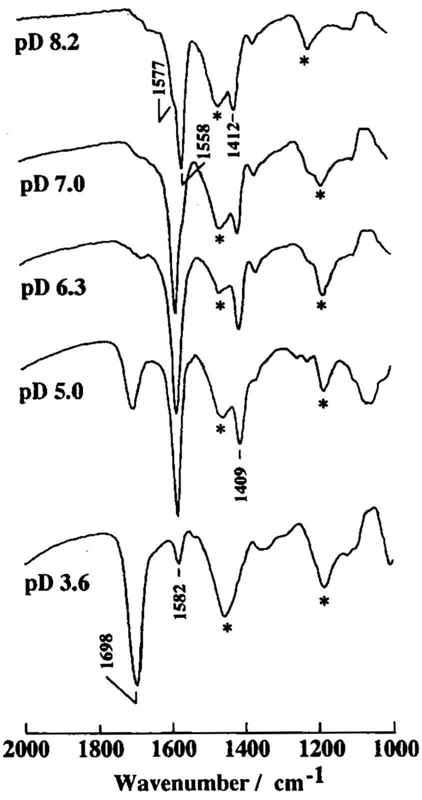


FIG. 2 IR spectra of CS in D_2O solution.
Peaks denoted by asterisks are due to solvents.

TABLE 1

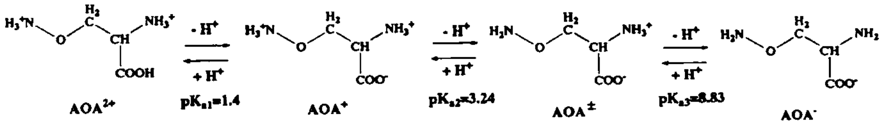
IR frequencies (cm-1) and band assignments of CS in aqueous solution

in H_2O, pH=			in D_2O, pD=			
3.6	6.2	8.0	3.6	6.3	8.2	Assignment
1715 s			1698 vs			ν(C=O), CS^+-I
1613 w	1613 sh					$\delta_a(NH_3^+)$, CS^+-I & $CS^\pm$-I
1573 w	1574 vs	1573 sh	1582 w	1581 s	1577 sh	$\nu_a(CON^-)$, $CS^\pm$-I
		1552 vs			1558 s	$\nu_a(CON^-)$, CS^-
1538 m	1532 sh					$\delta_s(NH_3^+)$, $CS^\pm$-I
	1410 w	1415 w		1409 m	1412 m	$\nu_s(CON^-)$, $CS^\pm$-I & CS^-

These two bands are ascribed to the antisymmetric and symmetric CON^- stretching modes, respectively, of the $CS^{\pm}$-I form. The existence of the $CS^{\pm}$-II form can be ruled out because no corresponding bands assignable to the cis amide are observed. When the pH is further increased, the 1573 cm^{-1} band decreases in intensity and is replaced by a new band at 1552 cm^{-1}. The 1552 cm^{-1} bands can be ascribed to the antisymmetric CON^- stretching vibration of the CS^- species. The corresponding symmetric mode is observed at 1415 cm^{-1}.

β-aminooxy-D-alanine

Scheme 3 shows the ionization equilibria of AOA. IR spectra at various pHs and pDs are shown in Figs. 3 and 4. Frequencies and band assignments are summarized in Table 2. The IR spectra of AOA are interpreted straightforwardly by referring to the data of α-amino acids[8]. At pH 0.1, the IR spectrum shows intense bands at 1740, 1616, 1540, 1255 and 1048 cm^{-1}. The 1740 and 1255 cm^{-1} bands are assignable to the C=O stretching and the C-O stretching modes of the AOA^{2+} form, respectively. As the pH is raised to 2.4, the 1616 cm^{-1} band assignable to the COO^- antisymmetric stretching of the AOA^+ species, where the NH_3^+ deformation band is overlapped, increases in intensity, while intensities of the 1740 and 1255 cm^{-1} bands observed for the AOA^{2+} species decrease drastically. The COO^- antisymmetric stretching band shifts to 1594 cm^{-1} at pH 5.4 and further to 1566 cm^{-1} at pH 12.1. These shifts are associated with deprotonation of the aminooxy (ONH_3^+) and ammonium (NH_3^+) groups, respectively. The low frequency shifts induced by the pH changes are clearly observed in D_2O solution.



Scheme 3 Ionic equilibria of AOA.

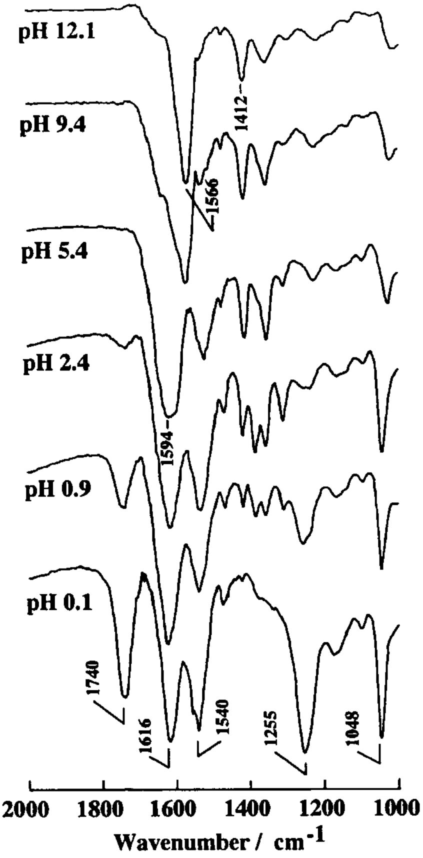


FIG. 3 IR spectra of AOA in H_2O solution.

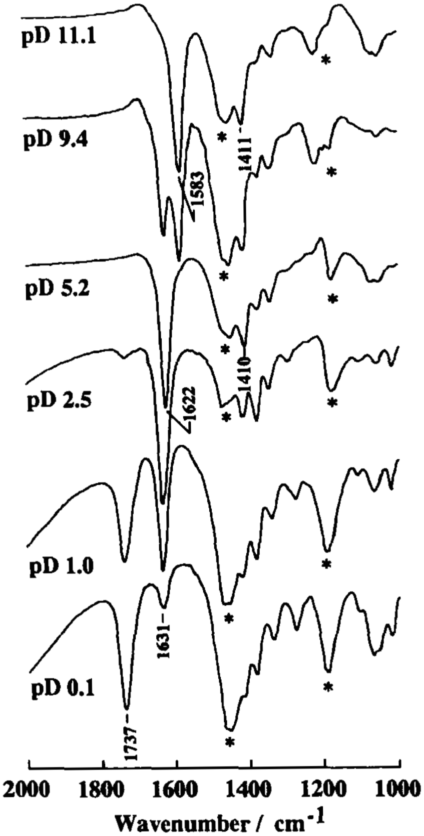


FIG. 4 IR spectra of AOA in D_2O solution.
Peaks denoted by asterisks are due to solvents.

CS-dimer

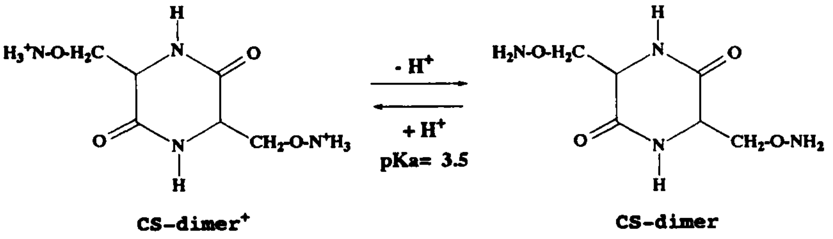
The ionic equilibrium of CS-dimer is shown in Scheme 4. This compound shows a single titratable group[9]. The pH- and pD- dependent IR spectra of CS-dimer are shown in Fig. 5 and the IR frequencies and band assignments are summarized in Table 3. The IR spectrum at pH 2.8 exhibits an intense peak at 1673 cm^{-1}

TABLE 2

IR frequencies and band assignments of AOA in aqueous solution

in H_2O, pH=				in D_2O, pD=				
0.1	2.4	5.4	12.1	0.1	2.5	5.2	11.1	Assignment
1740 s				1737 s				$\nu(C{=}O)$, AOA^{2+}
	1616 s			1631 vw	1631 s			$\nu_a(COO^-)$, AOA^+
		(1594)[a]				1622 s		$\nu_a(COO^-)$, $AOA^\pm$
			1566 vs				1583 s	$\nu_a(COO^-)$, AOA^-
1616 s	1616 s	1613 s		1467 s				$\delta_a(NH_3^+)$, AOA^{2+}
1540 s	1532 s	1519 m						$\delta_s(NH_3^+)$, AOA^{2+}, AOA^+ & $AOA^\pm$
	1417 w	1410 w	1412 w		1413 m	1410 m	1411 m	$\nu_s(COO^-)$, AOA^+, $AOA^\pm$ & AOA^-
1255 s								$\nu(C{-}O)_{carboxyl}$
1048 s	1044 m	1025 m						$\nu(C{-}O)_{aminooxy}$

[a] The band is overlapped with the 1613 cm^{-1} band.



Scheme 4 Ionic equilibrium of CS-dimer.

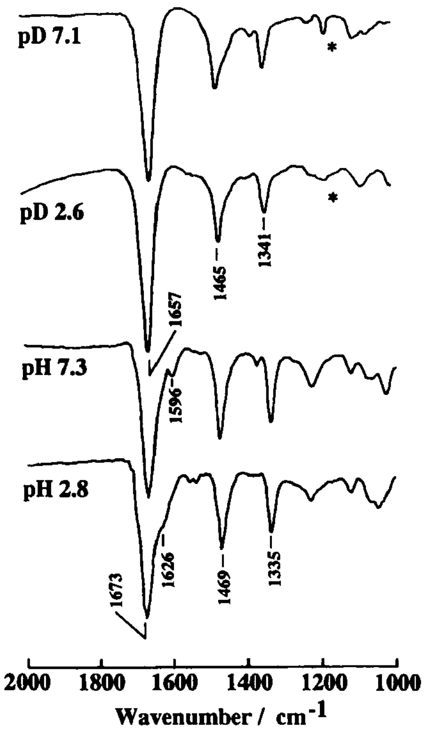


FIG. 5 IR spectra of CS-dimer in H_2O and D_2O solutions. Peaks denoted by asterisks are due to solvents.

TABLE 3

IR frequencies and band assignments of CS-dimer in aqueous solutions

in H_2O, pH=		in D_2O, pD=		
2.8	7.3	2.6	7.1	Assignment
1673 vs		1657 vs		ν(C=O), CS-dimer$^+$
	1667 vs		1650 vs	ν(C=O), CS-dimer
1626 sh				$\delta_a(NH_3^+)$, CS-dimer$^+$
	1596 vw			$\delta(NH_2)$, CS-dimer
1469 m	1470 m	1465 m	1469 m	$\delta(CH_2)$, CS-dimer$^+$ & CS-dimer
1335 m	1335 m	1341 w	1341 w	$w(CH_2)$, CS-dimer$^+$ & CS-dimer

(νC=O) and a shoulder at 1626 cm^{-1} ($\nu_a NH_3^+$), and medium bands at 1469 and 1335 cm^{-1}. When the pH is increased over the pKa, the 1673 cm^{-1} band shifts to lower frequency by 6 cm^{-1} and the 1626 cm^{-1} band disappears. On going from H_2O to D_2O solution, the ν(C=O) band undergoes lower frequency shift by 16 cm^{-1}, whereas the 1469 and 1335 cm^{-1} bands show little shift. These deuteration shifts are characteristic of the diketopiperazines[9].

Transformation of CS to CS-dimer in aqueous solutions

Fig. 6 shows the time dependent IR spectra of CS in 5 % D_2O solution at pD 6.2. It is noticed that the 1580 cm^{-1} band characteristic of the CS$^{\pm}$-I form decreases in intensity and new bands concomitantly appear with time at 1650, 1464 and 1342 cm^{-1} as time passes. IR frequencies of the latter three bands coincide with those of CS-dimer in D_2O solution. This spectral change indicates transformation of CS to CS-dimer. The spectral change is dependent on concentration of CS. The time dependence of IR spectra of CS were also

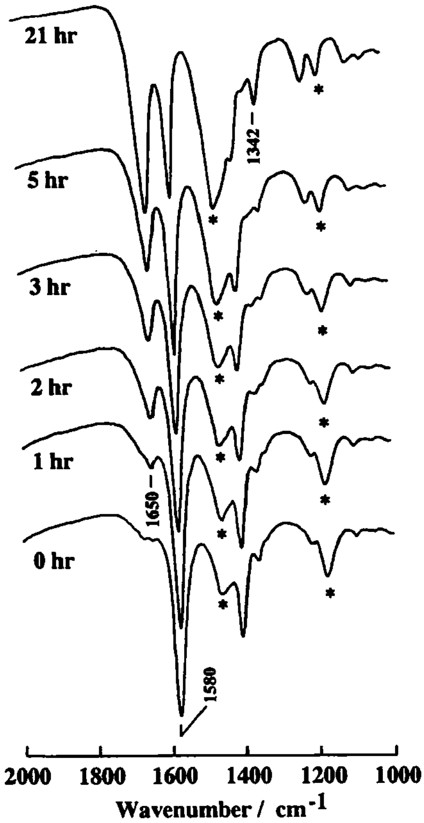


FIG. 6 Time dependence of IR spectra of CS in D_2O solution.
Peaks denoted by asterisks are due to solvents.

followed in D_2O solutions at pDs below the pK_{a1} and above the pK_{a2}. At pD below pK_{a1}, the C=O stretching mode of the CS^+-I at 1698 cm^{-1} decreases in intensity with time and a new band appears at 1633 cm^{-1}. The frequency of the new band coincides with the $\nu_a(COO^-)$ of β-aminooxy-D-alanine. Thus CS undergoes hydrolysis in solutions at pD below pK_{a1}, and no dimerization reaction is observed. At pD above the pK_{a2}, no spectral change is observed for the CS solutions.

The present findings show that spontaneous transformation of CS to CS-dimer occurs in aqueous solution and the process is pH dependent. It is conceivable that nucleophilic attack of the α-amino group of one CS molecule to the carbonyl group of the other is the first step of dimerization. The protonation at the α-amino group is, therefore, disadvantageous for the nucleophilic attack. Thus we assume that a non-ionic species $CS^{\pm}$-II takes part in the dimerization process, which is in equilibrium with the $CS^{\pm}$-I form. In this regard we have examined the effect of addition of less polar solvent on the rate of dimerization. The rate of dimerization is accelerated as an increasing content of methanol; it is doubled when 50 % of methanol is used as a solvent. IR spectra of CS in aqueous methanol solution exhibits a new band at 1690 cm^{-1}, in addition to the $\nu_a(CON^-)$ mode of the $CS^{\pm}$-I at 1574 cm^{-1}. The former band increases in intensity as increasing the content of methanol. This new band can be assigned to the cis amide I of the $CS^{\pm}$-II species, since N-acetylated CS (Ac-CS) shows an analogous amide I band at 1692 cm^{-1}. These observations lead to the conclusion that the non-ionic $CS^{\pm}$-II species is involved in the dimerization reaction.

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