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Spectroscopic studies of methylglyoxal in water and dimethylsulfoxide

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Abstract

Methylglyoxal is a highly reactive dicarbonyl compound, which reacts in vivo with biological macromolecules and thereby affects their structure and function. These changes are associated with complications during aging, diabetes and Alzheimer's disease as well as with growth inhibition in different tumors. Many enzymes are involved in the metabolism of methylglyoxal, but its true physiological role in metabolism and chemical properties are still obscure. In this study it was shown that methylglyoxal, during the freeze-drying of aqueous solutions, polymerizes into small polymeric structures which are stable in organic media such as dimethylsulfoxide. When re-exposed to water, the polymers are immediately transformed into the monomeric mono- and dihydrate forms of methylglyoxal. By NMR and UV spectroscopy, it was shown that solvent, temperature, and the amount of available water strongly influence the equilibrium of the different forms of methylglyoxal and thereby change its reactivity. ^{1}H and ^{13}C NMR spectroscopy were used to determine the structures of the different monomeric and oligomeric structures of methylglyoxal.

Keywords: Methylglyoxal; 1,1-Dimethoxypropanone; Methylglyoxal polymers

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1. Introduction

One of the important properties of carbonyl compounds, mostly aldehydes, is the formation of hydrates in aqueous solutions by nucleophilic addition of water to their carbonyl groups. The equilibrium among the free and hydrated forms of carbonyls and the dependency of that equilibrium on solvent, temperature, and other components in biological systems can play an important role in the chemistry of the compound and can affect other chemical processes occurring in those systems. Another important property of some aldehydes is their tendency to polymerize to form a variety of cyclic and acyclic structures. Simple aldehydes such as formaldehyde and acetaldehyde are well characterized in this respect. Endogenous dicarbonyl compounds like glyoxal and methylglyoxal also undergo these types of reactions. However, the presence of two carbonyl groups on two adjacent carbonyl atoms makes the reactions and equilibria more complex. The behavior of glyoxal in water has been the subject of numerous studies performed by NMR, FTIR, and UV spectroscopy [1–4], while corresponding spectroscopic data for methylglyoxal are limited.

Methylglyoxal is a physiological metabolite formed in vivo from numerous enzymatic and non-enzymatic reactions [5]. As a highly reactive α-ketoaldehyde it can react with biological macromolecules, proteins, nucleic acids, and lipoproteins changing their chemical and biochemical properties, and generating "advanced glycation end products" [6–8]. The level of methylglyoxal in cells is well controlled by the glyoxalase enzyme system, but fasting and metabolic disorders, such as diabetes, result in the accumulation of this toxic dicarbonyl compound [9]. However, despite nearly a century of research, the chemical properties of methylglyoxal and its true physiological role in metabolism are still unclear. To determine its role in various diseases many research groups have measured methylglyoxal levels and examined its biological effects in both in vitro and in vivo systems. For these studies, methylglyoxal was prepared following published procedures [5] or purchased from commercial sources. Investigations of the behavior of methylglyoxal were carried out only in aqueous solutions. The ^{1}H NMR spectrum of methylglyoxal in water has been reported but a detailed interpretation of the obtained results was not provided [10]. Moreover, the ^{13}C NMR spectrum of methylglyoxal has not been published. The probable cause for the absence of these data is its strong water affinity and ease of polymerization.

To address these deficiencies we investigated the properties of methylglyoxal in water and in dimethylsulfoxide. For the spectroscopic measurements we prepared aqueous solution of pure methylglyoxal by hydrolyzing 1,1-dimethoxypropanone in the presence of the ion exchange resin, Dowex-50×8 (H^+ form) which served as a catalyst [11]. To contribute to the understanding of its reactivity and molecular structure in different solvents, the prepared aqueous solution of methylglyoxal were freeze-dried and the qualitative composition of the residue was examined by UV and NMR spectroscopy in water and in dimethylsulfoxide solutions.

2. Materials and methods

Aqueous solutions of methylglyoxal were prepared in our laboratory according to the literature [11]. One- and two-dimensional homo- and heteronuclear NMR spectra were recorded on Varian Gemini 300 and Mercury 300 NMR spectrometers operating at 75.5 MHz for ^{13}C nuclei. The spectra were measured in D_2O and DMSO-d_6 solutions in 5 mm tubes at 20 °C. Chemical shifts in ppm were referenced to TMS in DMSO-d_6 and to dioxane in D_2O. The experiments carried out on the Varian Gemini 300 instrument were standard ^1H, ^{13}C broadband proton decoupled, ^{13}C gated proton coupled, correlation spectroscopy (COSY-45), long-range correlation spectroscopy (LRCOSY-45), and heteronuclear multiple-quantum coherence correlation spectroscopy (HETCOR) experiments. In ^1H and ^{13}C NMR spectra the digital resolution was 0.58 and 0.72 Hz, respectively. The experiments carried out on a Varian Mercury 300 instrument were standard ^1H, ^{13}C broadband proton decoupled, 1D nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple bond coherence (HMBC) experiments. The last two experiments were carried out with pulsed field z-gradient. In ^1H and ^{13}C NMR spectra measured with the Varian Mercury 300 MHz spectrometer the digital resolution was 0.33 and 0.55 Hz, respectively. All experiments were performed using Waltz-16 modulation. ^1H and ^{13}C assignments were made by a combination of ^1H–^1H and ^1H–^{13}C shift correlated 2D NMR techniques which include long-range ^1H–^1H COSY and ^1H–^{13}C HETCOR, combined with ^1H and ^{13}C correlation techniques through one bond (HSQC) and multiple bonds (HMBC). Absorption measurements were performed on a Varian Cary UV–visible spectrophotometer with a temperature regulated cell holder.

3. Results and discussion

3.1. Preparation of methylglyoxal and NMR analysis

Hydrolysis of freshly distilled 1,1-dimethoxypropanone (**1**) in water and in the presence of the ion exchange resin Dowex-50 × 8 (H^+ form) gave an aqueous solution of methylglyoxal [11]. The rate of hydrolysis was monitored by ^1H NMR spectroscopy. It was noticed that, in water, starting compound **1** existed in two different forms, while in organic solvent (DMSO-d_6) only one form was detected—the keto form (Table 1). In aqueous solution the second form could be the enol or the hydrate of compound **1**. We excluded the enol form on the basis of the multiplicity of the C-3 signal that shows quartet splitting in fully proton-coupled spectra. The distribution of the keto and hydrated forms for compound **1** was 64 and 36%, respectively, determined according to the integrated intensities of the resonances in the ^1H NMR spectrum.

After hydrolysis was complete, distillation of the unreacted starting material (i.e., **1**) and methanol (generated in the reaction) gave an aqueous solution of methylglyoxal (594 mM) in high yield (65%) [11]. The excess water was removed by freeze-drying and a light-yellow resin was obtained. Dissolving the resin in D_2O gave the ^1H

Table 1
^{1}H and ^{13}C NMR chemical shifts of 1,1-dimethoxypropanone (**1**) in D_2O and DMSO-d_6

Atom	Solvent	δ_H (ppm)		δ_C (ppm)	
		Keto	Hydrate	Keto	Hydrate
CH_3	DMSO-d_6	2.10	—	25.3	—
OCH_3		3.30	—	54.5	—
C=O		—	—	203.6	—
CH		4.55	—	103.5	—
C_q		—	—	—	—
CH_3	D_2O	2.04	1.11	26.3	22.1
OCH_3		3.21	3.34	60.7	57.3
C=O		—	—	209.3	—
CH		4.62	3.98	103.3	108.9
C_q		—	—	—	96.9

NMR spectrum shown in Fig. 1A, while dissolving the resin in DMSO-d_6 gave the spectrum shown in Fig. 1B. The large numbers of signals in these spectra are the result of polymerization of methylglyoxal during water removal and the existence of monomers of methylglyoxal in different forms. While the dimethylsulfoxide solution was stable, allowing the aqueous solution to stand at room temperature for several hours gave rise to pronounced changes in the spectrum. After 24 h, four main signals were observed (Fig. 1C).

The assignment of the methyl and methine protons was performed on the basis of long-range COSY spectra and confirmed by gradient HMBC experiments. In Fig. 2 bold numbers denote different forms of methylglyoxal (Fig. 3), while italic numbers represent particular couplings. In Table 2, the corresponding values of ^{1}H and ^{13}C NMR shifts are given. In HMBC spectrum residual one-bond C–H couplings in both forms are visible as satellite doublets centered on the corresponding proton signal (**2***1*

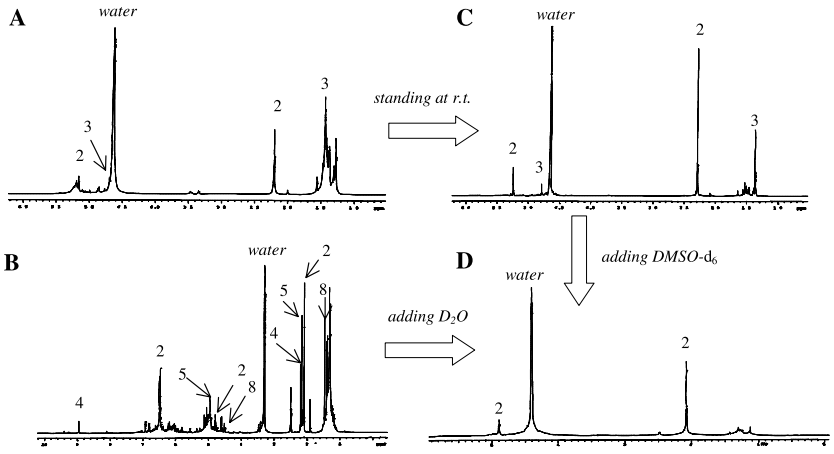

Fig. 1. ^{1}H NMR spectra of freeze-dried methylglyoxal sample; (A) in D_2O immediately after dissolving; (B) in DMSO-d_6; (C) in D_2O 24 h after dissolving; and (D) in D_2O:DMSO-d_6 1:1.

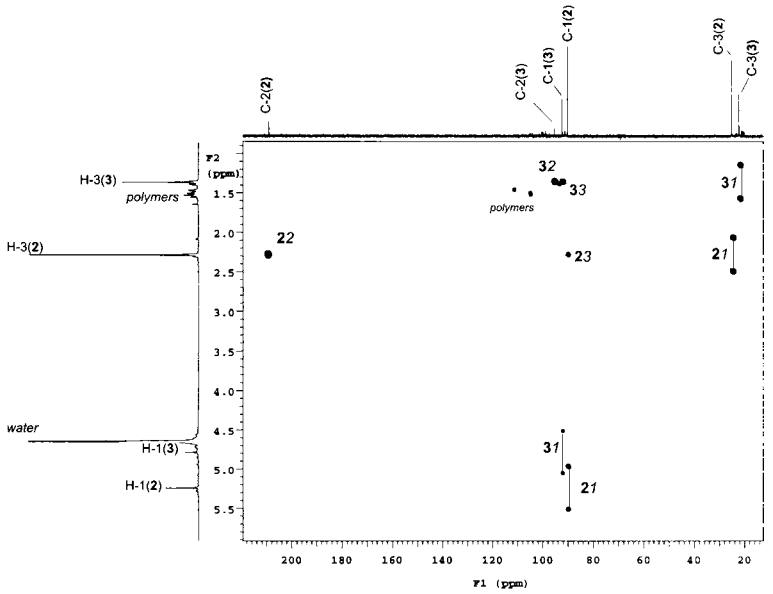

Fig. 2. Gradient HMBC spectrum of D_2O solution of freeze-dried methylglyoxal 24 h after dissolving in D_2O.

and **3***1*). The remaining cross signals belong to long-range C–H couplings in both species. The two-bond couplings between the methyl protons and the carbonyl C-atom in **2** at 209.9 ppm (**2***2*) and between the methyl protons and quaternary C-atom in **3** at 96.0 ppm (**3***2*), unambiguously show the presence of the mono- (**2**) and dihydrate (**3**) species of methylglyoxal in aqueous solution.

From the integrated signal intensities of the protons in monohydrate **2** and dihydrate **3** we obtained their ratio in the range of 56–62% for **2** and 38–44% for **3**, which is in agreement with the literature data [10]. 1D NOESY spectra confirmed the chemical exchange between the mono- and dihydrate forms in D_2O solution.

Adding water to the DMSO-d_6 solution of freeze-dried methylglyoxal caused the degradation of these polymer structures into monomeric methylglyoxal (Fig. 1D). Surprisingly, only one structure was detected, the monohydrate **2**. Adding more water did not cause the formation of dihydrate **3**. We assume that this phenomenon was the consequence of shifting the equilibrium between the mono- and dihydrate forms to the less hydrated species due to the presence of the organic solvent. To verify this, we added DMSO-d_6 into the aqueous solution and did not detect the appearance of any additional signals but did detect an increase in the monohydrate signal with a concomitant decrease in the dihydrate signal with every successive addition of the organic solvent. The monohydrate structure **2** was confirmed by its ^1H and ^{13}C chemical shifts (Table 2) and one-bond C–H coupling constants ($^1J_{CH} = 162.85 \pm 0.72$ Hz as compared to 163.57 ± 0.72 Hz in water).

The large number of signals in the ^1H NMR spectra of dimethylsulfoxide solutions of freeze-dried samples suggested a more complex mixture (Fig. 1B). It is

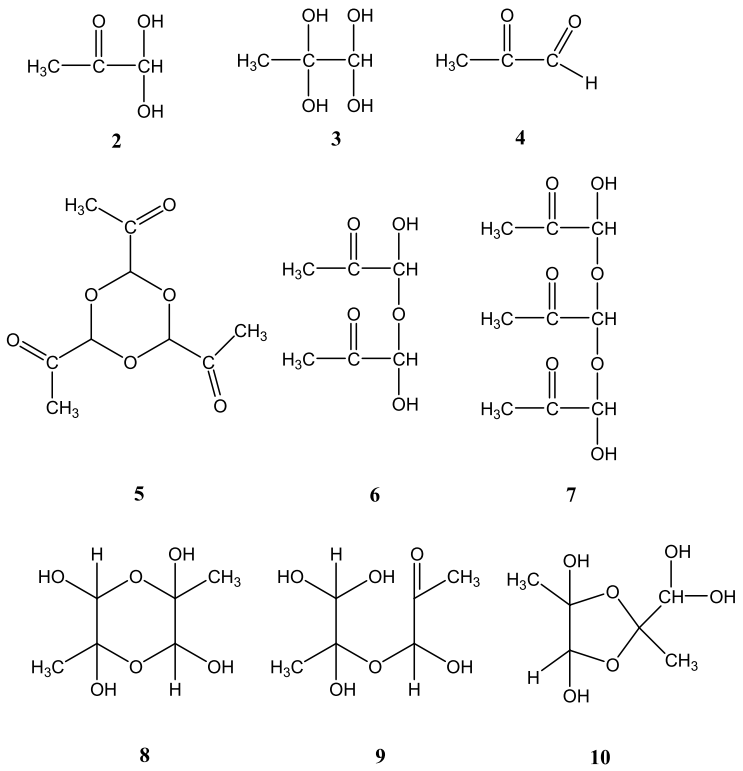

Fig. 3. Possible molecular species present in freeze-dried methylglyoxal.

obvious that methylglyoxal forms different monomeric and oligomeric structures (Fig. 3). In addition, structures **2**, **4**, **5**, **6**, **7**, and **9** could also participate in keto-enol tautomerization, thereby increasing the number of possible structures. The enol forms of all structures must have CH_2 protons. However, comparing the results obtained from HSQC and APT experiments did not confirm the presence of CH_2 protons: only CH and CH_3 protons were observed.

The keto-aldehyde form, **4**, is the most reactive isomer of methylglyoxal. Moreover, this isomer is processed by different enzymes such as the glyoxalase enzyme system, aldose reductase, betaine aldehyde dehydrogenase, and 2-oxoaldehyde dehydrogenase [12], resulting in its detoxification. However, **4** is also involved in many undesired reactions with proteins, nucleic acids, and lipoproteins, which result in the modification of the chemical and biochemical properties of these macromolecules [6–8]. The dicarbonyl form **4** is present in water only in traces [5], but we have detected a significant amount of it in dimethylsulfoxide solution. The presence of **4** in this mixture is supported by a signal at 8.97 ppm, which is typical for aldehyde protons. In the HMBC spectrum, residual one-bond C–H coupling (**4***1*) between the aldehyde proton and the carbonyl C-atom, as well as two-bond C–H coupling (**4***2*) with another carbonyl C-atom, support the assigned structure of **4** (Fig. 4C).

Table 2
^{1}H and ^{13}C NMR chemical shifts of different forms of methylglyoxal

Atom	Structure	Chemical shifts	
		δ_H (ppm)	δ_C (ppm)
CH_3	**2**	2.30	25.4
CH		5.25	90.6
C=O		—	209.9
C_q		—	—
CH_3	**3**	1.19	22.2
CH		4.30	92.7
C=O		—	—
C_q		—	96.0
CH_3	**4**	2.22	22.9
CH		8.97	188.8
C=O		—	197.3
C_q		—	—
CH_3	**5**	2.17	24.2
CH		5.07	100.9
C=O		—	205.7
C_q		—	—
CH_3	**8**	1.49	14.0
CH		4.63	98.0
C=O		—	—
C_q		—	106.8

Moreover, the long-range couplings of the methyl protons at 2.22 ppm with the carbonyl atoms further confirmed the structure of **4**. We also observed that warming DMSO-d_6 solutions up to 70 °C increased the relative level of **4**. In Table 2, the ^{1}H and ^{13}C chemical shifts of **4** are given.

No amount of dihydrate **3** was detected in dimethylsulfoxide solution, while the monohydrate form **2** was established by one-bond and long-range C–H couplings (Fig. 4). The ^{1}H and ^{13}C chemical shifts of **2** are in agreement with those found in D_2O solution. In contrast to the behavior in D_2O, long-range couplings between the hydroxyl hydrogens and the methyl (**2***4*) (Fig. 4A) and the methine (**2***2*) (Fig. 4B) carbons were observed in DMSO-d_6 solution. In addition, the methine proton appeared as a triplet due to spin–spin coupling with two neighboring hydroxyl hydrogens ($J = 6.51$ Hz, average value). By adding small amounts of D_2O, the hydroxyl protons disappeared and the CH proton of **2** changed to a singlet. These observations also confirm the structure of **2**.

Residual one-bond C–H couplings (**5***1*) of the methyl (Fig. 4A) and the methine groups (Fig. 4B), as well as two-bond couplings (**5***2*) between the methyl and methine groups and the carbonyl C-atom at 205.7 ppm (Fig. 4C) enabled the characterization of trioxane **5**. The dimeric and trimeric structures, **6** and **7**, were probably present in the mixture, because trioxane **5** likely forms from **6** and **7**. However, due to their low concentrations and overlapping signals it was not possible to characterize them.

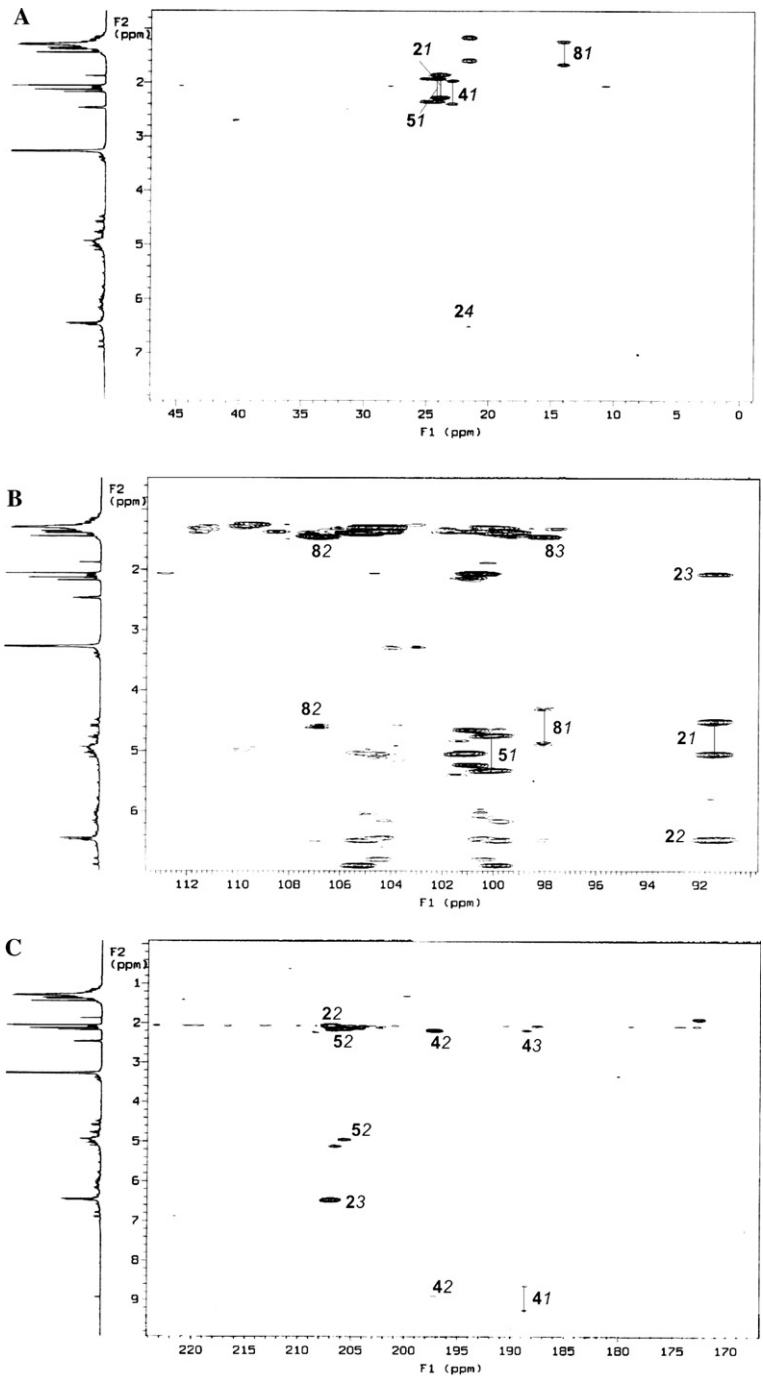

Fig. 4. Typical regions of the gradient HMBC spectrum of DMSO-d_6 solution of freeze-dried methylglyoxal.

The dimeric structure **9** could be formed by nucleophilic addition of the hydroxyl group of monohydrate **2** to the carbonyl group of **4**. In structure **9**, an analogous intramolecular reaction could follow, which should result in a six-member dioxane ring **8** or a five-member dioxolane ring **10**. The fact that structures **9** and **10** can exist as four diastereomers, and structure **8** can exist as five different diastereomers, complicates their identification in the mixture due to small differences in their chemical shifts. Overlapping of the hydroxyl proton signals also impedes determination of the coupling constants of these protons with the corresponding CH protons. Hence, identification of the different diastereomeric forms was not possible. One-bond C–H couplings (**8***1*) of the methyl (Fig. 4A) and the methine groups (Fig. 4B), as well as the long-range couplings (**8***2*) between these groups and the quaternary C-atom at 106.8 ppm (Fig. 4B) may be indicative of one out of five possible stereoisomers of the dimeric dioxane structure **8**.

In summary, the ^{1}H and ^{13}C NMR analysis confirmed the existence of different monomeric and oligomeric structures of methylglyoxal in solution. The significant influence of water and organic solvent on the equilibrium of these different forms has also been demonstrated.

3.2. UV analysis of freeze-dried methylglyoxal in water and organic solvents

The solvent effects on methylglyoxal were also investigated by using UV–Vis spectrophotometry (Fig. 5). A broad absorption peak at 290 nm, corresponding to the n to π^* excitation band, a characteristic of carbonyl compounds, was observed in the three solvents examined: water, DMSO and dioxane (Figs. 5A–C).

The lowest-energy absorption band centered at 430 nm, a characteristic of 1,2-dicarbonyl compounds, was not observed in water or in organic solvents. This indicates that only a negligible fraction of the free form of methylglyoxal **4** is present. The UV–Vis spectra in DMSO solution, taken every 30 min during a 2.5 h period at 60 °C, showed an increase of the intensity of the band at 290 nm, with no absorption at 430 nm (Fig. 5D). The increase at 290 nm indicates changes in the equilibria among the different carbonyl forms of methylglyoxal with temperature. However, dehydration of organic solutions of methylglyoxal, performed by activated molecular sieves over a 3 day period, gave rise to the appearance of an absorption band at 430 nm (Fig. 5E, upper curve). The band completely disappeared upon the addition of a few drops of water (Fig. 5E, lower curve), indicating again the significant influence of water on the equilibria of the different forms of methylglyoxal.

4. Conclusion

By NMR and UV spectroscopy it has been shown that methylglyoxal can change from the less reactive non-carbonyl form to a more reactive carbonyl and dicarbonyl forms, and vice versa. These changes are dependent on the solvent, temperature, and the amount of water present. It is well known that methylglyoxal in living cells is

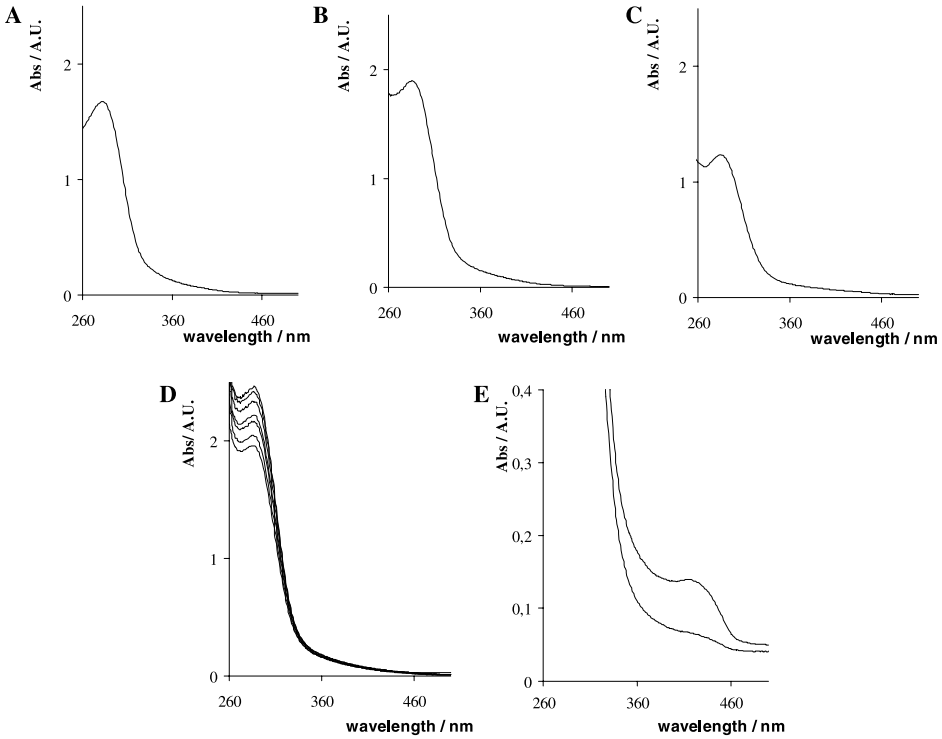

Fig. 5. UV spectra of freeze-dried methylglyoxal in: (A) water (8.3 mg/ml); (B) dioxane (8.3 mg/ml); (C) DMSO (12.5 mg/ml); (D) the solution in (C) at 60 °C with spectra collected every 5 min during 30 min; and (E) the solution in (C) after standing 3 days over molecular sieves (upper curve) and after the addition of a few drops of water to the solution (lower curve).

generated from different sources [5]. Living cells represent markedly complex and changeable heterogeneous media with different amounts of water in hydrophilic and hydrophobic regions. Thus, different reactivities of methylglyoxal in the various parts of a cell are expected. In addition, the presence of different organic components in aqueous cytoplasm could influence methylglyoxal reactivity. From these observations it is suggested that different parts of cells or tissues could be more sensitive to modifications by methylglyoxal.

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