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ORGANIC SULFUR COMPOUNDS RESULTING FROM THE INTERACTION OF IRON SULFIDE, HYDROGEN SULFIDE AND CARBON DIOXIDE IN AN ANAEROBIC AQUEOUS ENVIRONMENT

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Abstract. The reaction of iron sulfide (FeS) with H₂S in water, in presence of CO₂ under anaerobic conditions was found to yield H₂ and a variety of organic sulfur compounds, mainly thiols and small amounts of CS₂ and dimethyldisulfide. The same compounds were produced when H₂S was replaced by HCl, in the H₂S-generating system FeS/HCl/CO₂. The identification of the products was confirmed by GC-MS analyses and the incorporation of H₂ in the organic sulfur compounds was demonstrated by experiments in which all hydrogen compounds were replaced by deuterium compounds. Generation of H₂ and the synthesis of thiols were both dependent upon the relative abundance of FeS and HCl or H₂S, i.e. the FeS/HCl- or FeS/H₂S-proportions. Whether thiols or CS₂ were formed as the main products depended also on the FeS/HCl-ratio: All conditions which create a H₂ deficiency were found to initiate a proportional increase in the amount of CS₂. The quantities of H₂ and thiols generated depended on temperature: the production of H₂ was significantly accelerated from 50 °C onward and thiol synthesis above 75 °C. The yield of thiols increased with the amount of FeS and HCl (H₂S), given a certain FeS/HCl-ratio and a surplus of CO₂. A deficiency of CO₂ results in lower thiol synthesis. The end product, pyrite (FeS₂), was found to appear as a silvery granular layer floating on the aqueous surface. The identity of the thiols was confirmed by mass spectrometry, and the reduction of CO₂ demonstrated by the determination of deuterium incorporation with DCl and D₂O. The described reactions can principally proceed under the conditions comparable to those obtaining around submarine hydrothermal vents, or the global situation about 4 billion years ago, before the dawn of life, and could replace the need for a reducing atmosphere on the primitive earth.

1. Introduction

The perspective that the advent of organic compounds on earth may have occurred in iron- and sulfur-rich environments such as submarine hot springs, has certainly grown in recent years. The presence of organic compounds, iron, sulfides and pyrite in submarine hot springs (Cairns-Smith *et al.*, 1992; Holm, 1992; Larter *et al.*, 1981; Russell *et al.*, 1988) have been treated extensively, as has the link between the iron- and sulfur-cycles (Zhuang *et al.*, 1992). Interest in iron-sulfur compounds has also included their involvement in processes possibly contributing to the origins of life (Ferris, 1992; Holm, 1992, Holm and Hennes, 1992; Popper, 1990; Russell *et al.*, 1988; Russell *et al.*, 1990; Williams, 1990; Yanagawa and Kobayashi, 1989), especially the abiotic synthesis of amino acids (Hennes *et al.*, 1992; Holm and Andersson, 1994). Concerning the theoretical background, two models were of

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special interest within this realm: de Duve's thioester world and Wächtershäuser's surface metabolism. The thioester model, which really is a thioester-iron world (de Duve, 1991, 1992), is based on "the presence of thiols and carboxylic acids" (de Duve, 1991), but does not answer the question, how the required reduced carbon compounds have been synthesized. Alternatively, Wächtershäuser has propagated his theory of surface metabolism (1988, 1990a,b, 1992, 1994), which postulated the formation of organic material from the reaction of iron sulfide with hydrogen sulfide and carbon dioxide. In this context Drobner *et al.* (1990), have shown that the hydrogen required for CO₂ reduction is evolved from these reactions, although at very high concentrations of H₂S, and that these can also elicit the formation of ammonia, ethane, ethene, and acetic acid, the latter from thioglycolic acid (Blöchl *et al.*, 1992). Kaschke *et al.* (1994) have furthermore demonstrated the reducing capability of the FeS/FeS₂-system with cyclohexanone as a model compound. Against this background and in view of Wächtershäuser's postulations, we have examined the aqueous anoxic systems FeS/H₂S/CO₂ and FeS/HCl/CO₂ and report here on the formation of hydrogen and the time- and temperature-dependent synthesis of thiols and other sulfur compounds.

2. Experimental Procedures

60-ml serum bottles, sealed with Al-capped silicone stoppers, containing 10–150 mg (0.114–1.71 mmol) FeS in 10 ml H₂O were supplied with an N₂/CO₂ atmosphere (80:20, 130 kPa) or a 100% CO₂ gas phase by repeatedly degassing and flushing with the desired gas. The FeS routinely used was from Aldrich (99.9% purity), but as control experiments FeS from other sources (Alfa and Johnson Matthey, both 99.9% pure) was applied. The bottles were charged with a 1–10 ml (20–200 μ mol) H₂S-injection, prepared by adding 50% H₂SO₄ to evacuated serum bottles with Na₂S \times 9H₂O. The pH of this system was about 4.6. When the H₂S-generating system was used instead of H₂S, the volume was reduced to 9 ml and 1 ml HCl of a given concentration was added to start the reaction. In this system the final pH was approximately 3.6. The reactants were kept at a given temperature (mostly 90 °C) for a given time (days). Headspace samples were taken with a Monoject syringe equipped with a Teflon piston and passed through an 80 mm-length, 4-mm inner diameter tube containing 200 mg of Tenax GC (80/100 mesh) and flushed with a fixed volume N₂ gas to expel excessive H₂S, which would otherwise interfere with the first peak(s). The special design of the inlet system (Derikx *et al.*, 1990) of the Packard 438 A gas chromatograph (with a flame photometric detector) allowed replacement of a Tenax tube within seconds, and analysis were carried out (system I) on a Carboxen B HT (40/60 mesh) 2.0 m glass column (6.0 mm outer and 4.0 mm inner diameter) with 90 ml/min N₂ flow; detector and injector temperature was 190 °C, temperature program 1 min initial temperature (80 °C), then 30 °C/min rise to 140 °C. To confirm the identification of the compounds, analyses were also

done (system II) with 20% SE-30 on a Chromosorb PNAW (60/80 mesh) 1.50 m glass column (6.0 mm outer and 4.0 mm inner diameter) with 90 ml/min N_2 flow; detector and injector temperature was 190 °C, temperature program 3 min initial temperature (50 °C) then 35 °C/min rise to 190 °C. All data were calculated for a 50 ml headspace, and the partial removal of methanethiol by flushing with N_2 , which had been previously determined, was taken into account. The thiols and other sulfur compounds used as standards and for calibration were from Aldrich, 97–99% purity.

In addition to the GC determinations, headspace samples were analysed with a GC-MS-installation equipped with an inlet system for Tenax tubes: Mass spectrometer Finnigan MAT TSQ-70 (triple-quad) EI (electron impact) mode, scan mass 30 \rightarrow 100 1 sec/scan. GLC Hewlett-Packard model 5890, column HP5 50 m, 0.32 mm inner diameter, carrier gas Helium, column temperature isotherm 50 °C. Analyses were carried out by Mr. N. Bouter at the Analytical research department of Quest International at Naarden, with headspace samples containing about 250–300 ng thiols. To determine the identity of the compound with the molecular weight 62, the isomers ethanethiol and dimethyl-sulfide were separately co-chromatographed with otherwise identical samples. The compound was this way identified as ethanethiol.

In order to demonstrate the incorporation of hydrogen into the organic compounds, experiments were done with D_2O (Aldrich, 99.9% D_2O) as the water phase and DCl (Aldrich, 37% DCl in D_2O) replacing HCl . All other conditions were not altered and samples were analyzed on the GC-MS installation described above. Additional analyses were done with a Varian Saturn GC/MS, column DB 5 30 m, 0.25 mm inner diameter, temperature program 80–140 °C, at the Dept. of Organic Chemistry at the University of Nijmegen.

Determination of H_2S was done with system I at 80 °C. Hydrogen was determined on a Hewlett Packard 5890 A GC, equipped with a thermal conductivity detector and a column packed with Poropack Q (80-100 mesh), oven temperature 40 °C. When H_2S was replaced by HCl , the other conditions were not altered.

3. Results

The project was initiated by following the generation of H_2 while FeS , H_2S and CO_2 were allowed to react together at 75 °C (under anaerobic conditions) for at least one week. Gas-chromatographic analysis of the organic material resulting at the end of this time revealed the presence of organic sulfur compounds (OSC). It was then demonstrated that they could be detected after much shorter reaction times (Table I), It was found that about 50 nmoles OSC are formed after ca. 2 days, consisting of a mixture of 7 identified and 2 unidentified thiols, CS_2 and dimethyldisulfide (DMDS). The main product was shown to be methanethiol, together with ethanethiol and much smaller amounts of propane-, butane- and pentane-thiols. Carbon disulfide (CS_2) and dimethyldisulfide (DMDS) accounted

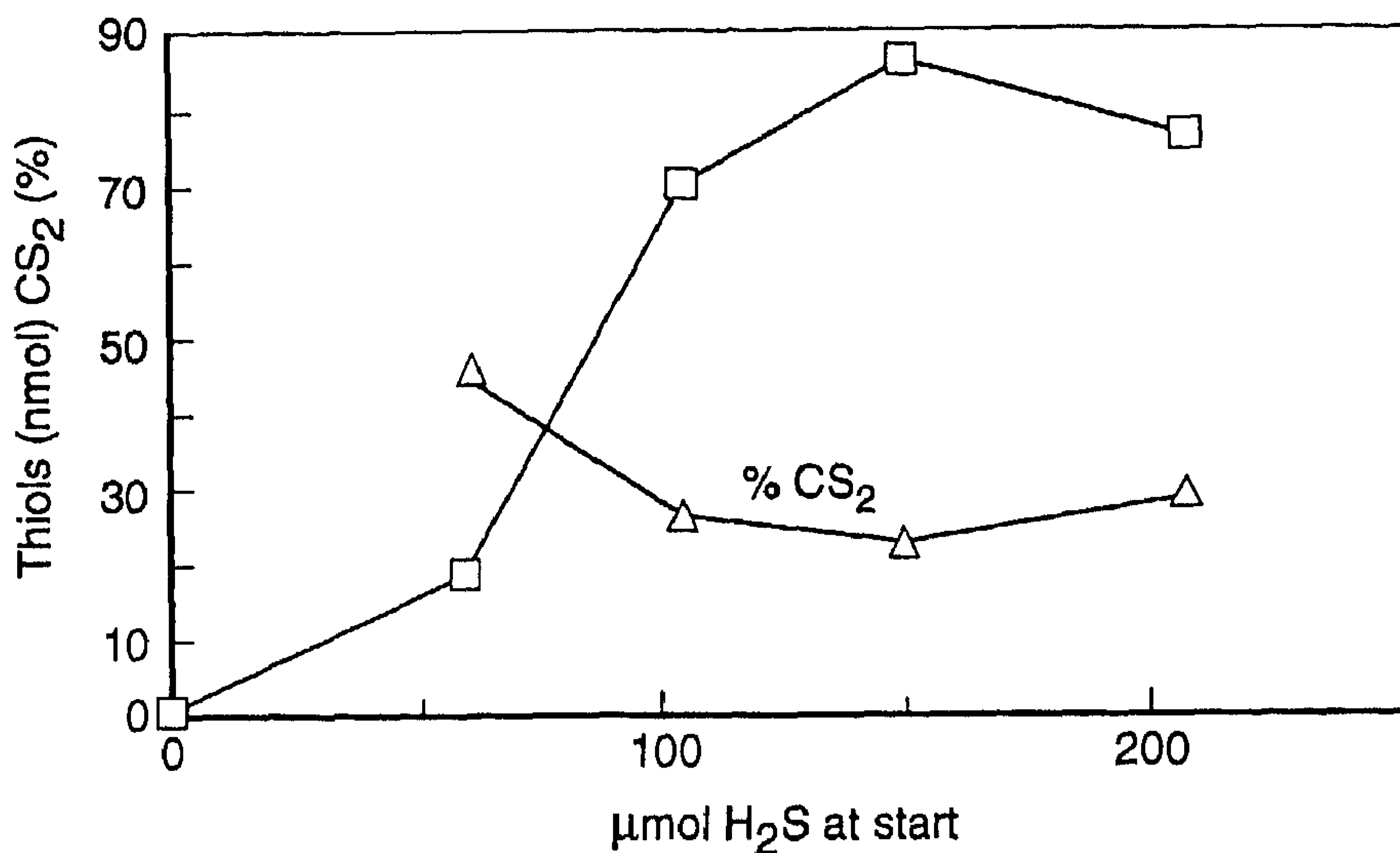


Figure 1. Effect of H_2S variations on the synthesis of thiols (nmol, squares), and the occurrence of CS_2 (percent of total OSC, triangles) from $570 \mu\text{mol FeS}$. (Values for H_2 were below $0.5 \mu\text{mol}$).

for 2.3% of the total of OSC. Control experiments showed that only negligible amounts of OSC are detectable if H_2S is omitted from the system. In the absence of FeS ("only H_2S ") generation of H_2 did not occur, and the quantity of OSC was also again very low. In both control experiments the very low yield did not increase with prolonged reaction times of up to 2 weeks.

Thiol synthesis was found to increase linearly with the amount of FeS and time within certain limits. The optimum amount of H_2S has been determined with various FeS concentrations, and a molar ratio $\text{FeS} : \text{H}_2\text{S}$ of $\pm 4:1$ was found to give maximum yields of thiols (Figure 1). Below and above this optimum, the diminished thiol synthesis was accompanied by an increased CS_2 formation.

From several other parameters examined, it was demonstrated that lowering of the pressure resulted in an increase of OSC in the headspace with a corresponding decrease in the aqueous phase. This suggested that the reactions would also proceed under normal pressure. Altering the pH in the reaction bottles led to an increase of thiol formation in the acid region and a decrease beyond pH 8, most probably due to the formation of increasingly more water soluble RS^- and thiolates. We observed furthermore the appearance of a silvery granular layer which floated on the aqueous phase. It was identified (X-ray diffraction) as FeS_2 , consisting mainly of (cubic) pyrite together with smaller amounts of (rhombohedral) marcasite (Figure 2). The development of the "floating" FeS_2 was visibly affected by time, temperature and the availability of both FeS and H_2S . It was not formed in absence of injected H_2S , a condition where H_2 generation occurs, but OSC are produced

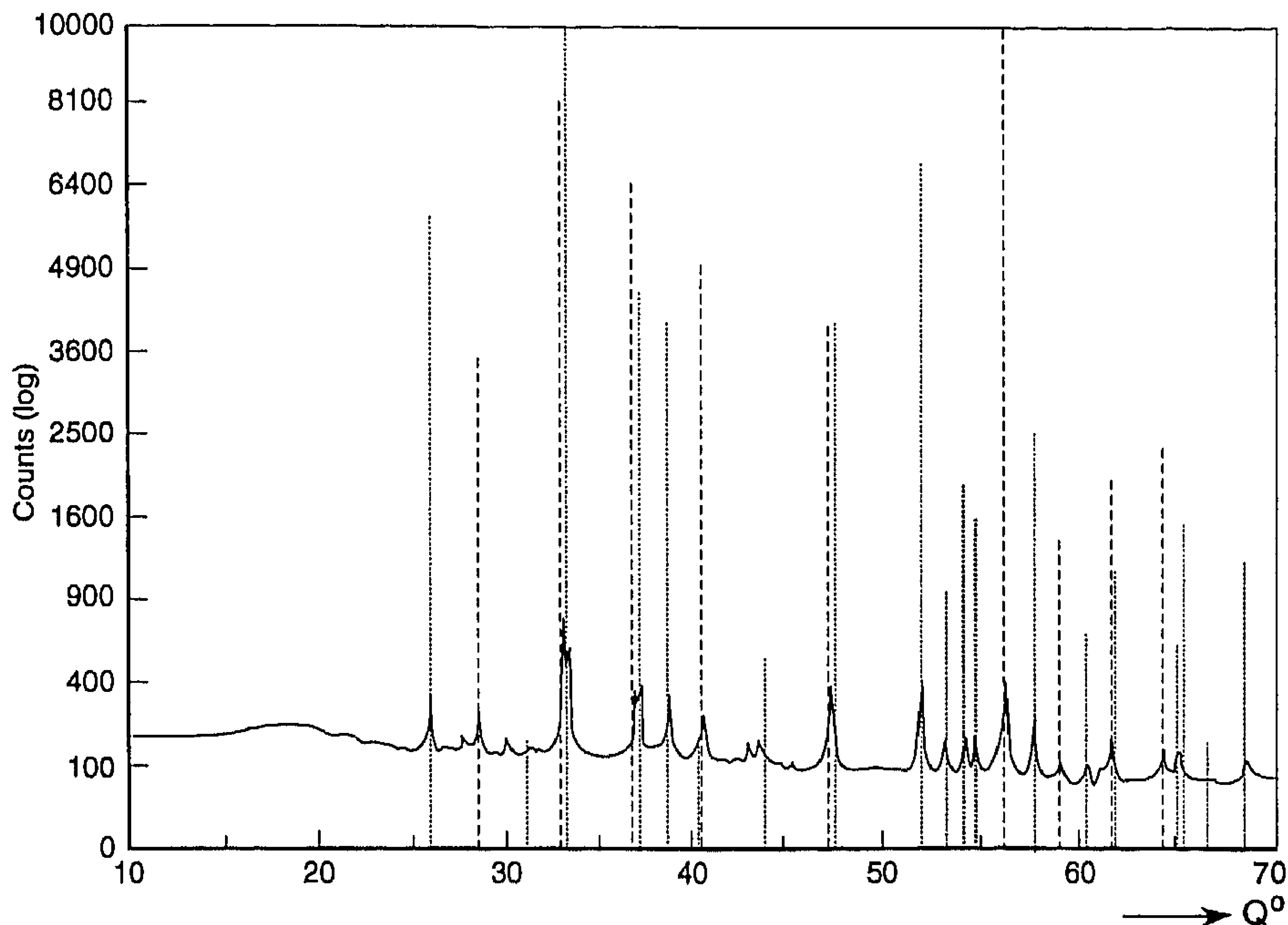


Figure 2. X-ray powder diffraction pattern of the material developing as a “floating” surface layer on the aqueous phase during the FeS/H₂S (HCl)/CO₂ reaction (solid line). Analyses were performed on a Philips PW 1710 diffractometer with a Cu LFF monochromator and compared with authentic spectra from data files (vertical lines).

only in insignificant amounts. The occurrence, development and properties of the “floating” FeS₂-layer, which precipitates immediately upon the addition of organic solvents, were determined by several other parameters (unpublished data), which suggested a binding of organic material to the mineral surface (e.g. CH₃S[−]), as proposed by Wächtershäuser (1990b) and observed with several iron-sulfur minerals (Holm *et al.*, 1993) and the self-assembly of thiols on Au surfaces (Fenter *et al.*, 1994).

The FeS/H₂S/CO₂-system can be varied by replacing H₂S by HCl, thus changing to the H₂S generating system FeS/HCl/CO₂. The same compounds as listed in Table I were obtained with this system (Figure 3).

Formation of H₂ and OSC with the FeS/HCl/CO₂-system was found to be affected by the FeS/HCl ratio and reaction time. Increasing amounts of FeS+HCl (in a constant ratio) were found to prolong the reaction time (Figure 4): With 50 mg FeS (0.57 mmol) and 0.65 mmol HCl, maximum production of thiols was reached after 5 days, with 100 mg FeS (1.14 mmol) and 1.3 mmol HCl maximum production was observed after 9–10 days. In all cases H₂S, formed primarily from FeS+HCl, was found to decrease with time.

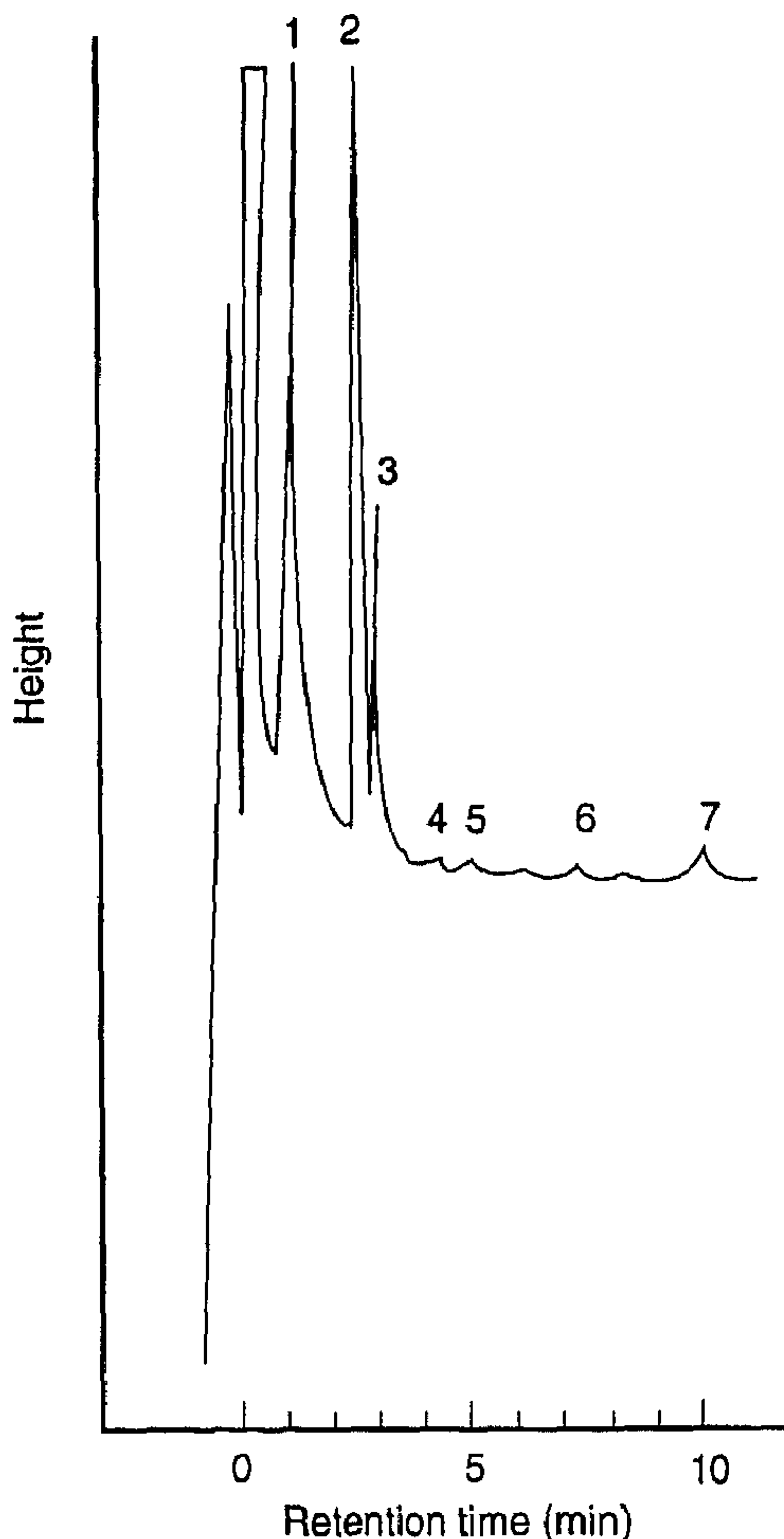


Figure 3. Representative GC trace of 1 ml headspace from a bottle with 50 mg FeS (0.517 mmol) and 0.65 mmol HCl. The H_2S peak (off-scale, r.t. 0.45) is followed by (1) methanethiol, (2) ethanethiol, (3) carbon disulfide, (4) isopropanethiol, (5) propanethiol, (6) DMDS and (7) butanethiol. One minute after start begins the temperature rise from 80 °C to 140 °C.

When product formation was followed during the first 12 h (Figure 5), it was found that H_2S formation increased first sharply, then more slowly up to 10 h, whereafter it began to decrease. Production of thiols began to increase after 2 h, and H_2 formation started simultaneously but stayed below 2 μmol , most probably because it is immediately consumed for CO_2 reduction in this early stage of thiol synthesis.

The ratio of FeS to HCl was also found to determine the proportional amounts of the end products. With (relatively) high amounts of FeS and low amounts of H_2S (and *vice versa*) the proportion of CS_2 increased drastically while it never made up

Table I

Organic sulfur compounds resulting from the reaction of FeS with H₂S and CO₂ at 75 °C after 2 days (headspace samples, GC-system I).

Retention time (min)	Compound (accor- ding to standards)	nmol	% of total	Controls (nmol)	
				FeS only	H ₂ S only
1.15	methanethiol	31.17	63.6	0.02	0.29
2.65 ^a	ethanethiol	13.53	27.6	0	0.02
3.12	carbon disulfide	0.93	1.9	0.11	0.43
4.45	isopropanethiol	0.12	0.2	0	
5.25	propanethiol	2.04	4.2	0	
7.18	dimethyldisulfide	0.18	0.4	0.01	0.06
8.45	sec. butanethiol	0.11	0.2	0	
10.02	butanethiol	0.63	1.3	0	
22.31	pentanethiol	0.28	0.6	0	
	Total (nmol)	48.98		0.14 ^b	0.80 ^b

^a The retention times of ethanethiol and dimethylsulfide (DMS) were too close to be discernable, but upon addition of KOH the peak of RT 2.65 vanished (together with the other thiol peaks), due to the formation of water soluble thiolates, which thus disappear from the headspace. In contrast, the DMS (and DMDS) -peaks were not altered by this treatment in control experiments with the pure compounds.

^b The value remained unaltered with prolonged reaction times. The compounds in the controls (mainly CS₂) did not originate from the silicone stoppers used throughout these experiments.

Table II

Formation of CS₂ (in % of total OSC) with various combinations of FeS and HCl at 90 °C (left part) and at different times and temperatures (right part, with 0.855 nmol FeS and 1.0 mmol HCl)

HCl mmol	FeS (mmol)			Time (h)	CS ₂ (%)	
	0.57	1.14	1.71		22 °C	90 °C
0.10	22.0	—	52.7	3	49.3	12.0
0.15	4.2	22.7	37.0	5	17.2	7.6
0.25	2.5	7.3	10.0	7	—	5.8
0.30	1.9	3.2	5.0	22	9.8	2.1
0.50	2.4	2.5	3.0	30	3.2	2.6
1.10	3.8	2.7	2.3	72	3.5	2.7
1.60	14.0	10.7	1.8	120	4.9	2.8
2.30	35.5	—	3.5			
3.00	58.3	24.8	12.7			

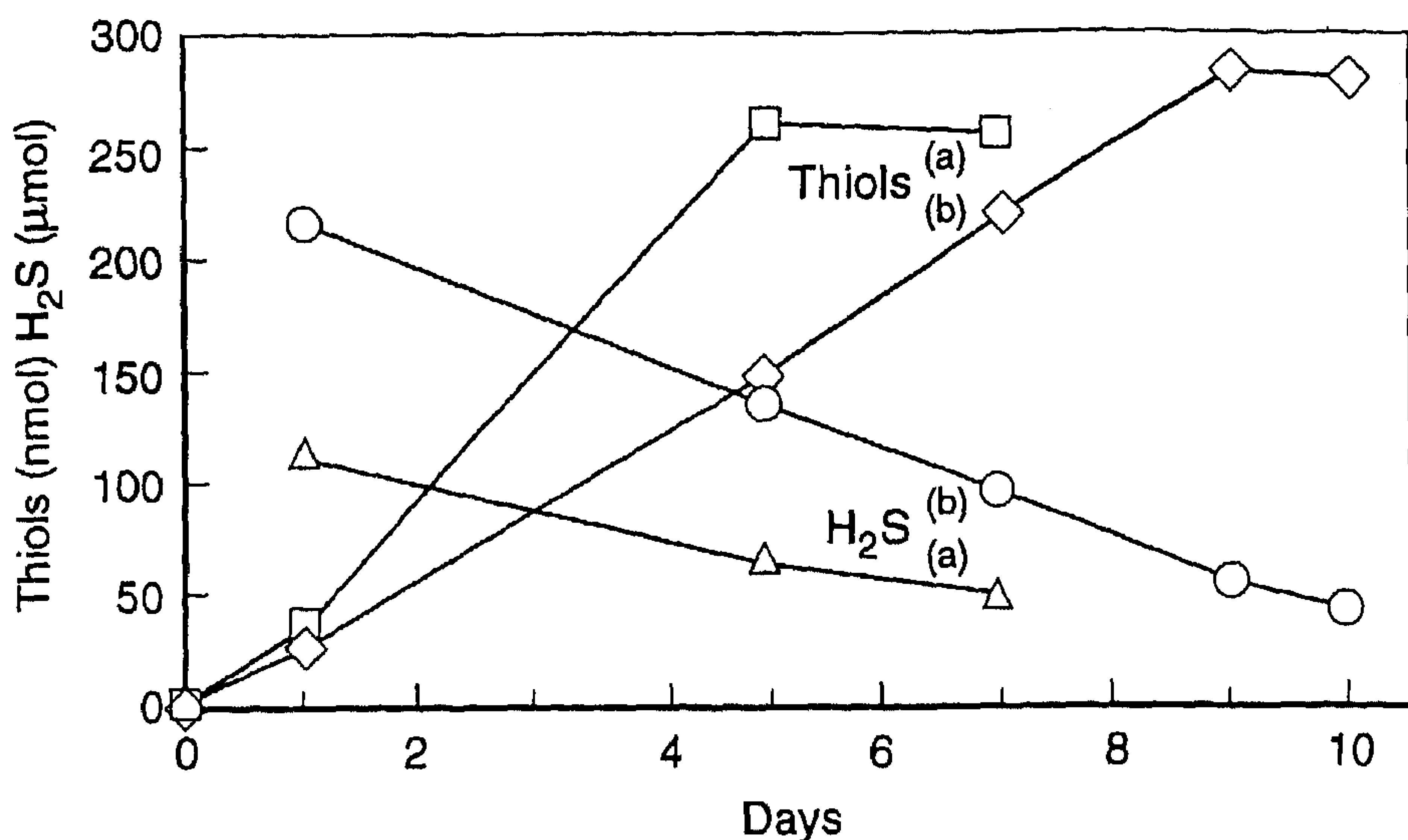


Figure 4. Effect of reaction time on the formation of (a) thiols (squares) and H₂S (triangles) with 0.57 mmol FeS and 0.65 mmol HCl, and (b) with 114 mmol FeS (diamonds = thiols, circles = H₂S) and 1.3 mmol HCl at 90 °C. Here and in all following graphs H₂S (and/or H₂) in μmol, thiols in nmol.

Table III

Thiol synthesis (all values in nmol) with various combinations of FeS and HCl with an N₂/CO₂ (=20% CO₂) gas phase (upper part) and with a 100% CO₂ gas phase (lower part)

Gas phase	FeS (μmol)	day	HCl (μmol)					
			165.2	325	650	1000	1300	2000
N ₂ /CO ₂ =20% CO ₂	285	3	77.3	129.3	108.6			
	570	5	170.2	220.4	260.0	214.5	173.1	
	855	7		150.2	195.2	223.2	218.1	130.2
	1140	10			235.7	252.0	258.1	195.5
100% CO ₂	285	3	78.2	128.4	103.6			
	570	5	167.8	218.7	263.1	213.8	176.2	
	855	7		170.7	316.6	358.1	258.7	147.1
	1140	10			325.4	395.3	436.2	227.1

more than 2–3% of the total OSC with better balanced FeS/HCl ratios (Table II, left part). The reaction time and temperature (see below) also had an effect on CS₂ formation (Table II): High proportions of CS₂ were observed as well at the very start of the reaction, and at low temperatures.

Combination of various amounts of HCl and FeS revealed optimum FeS/HCl ratios for both H₂ and thiol formation (Figure 6a and b, and Table III). Based on

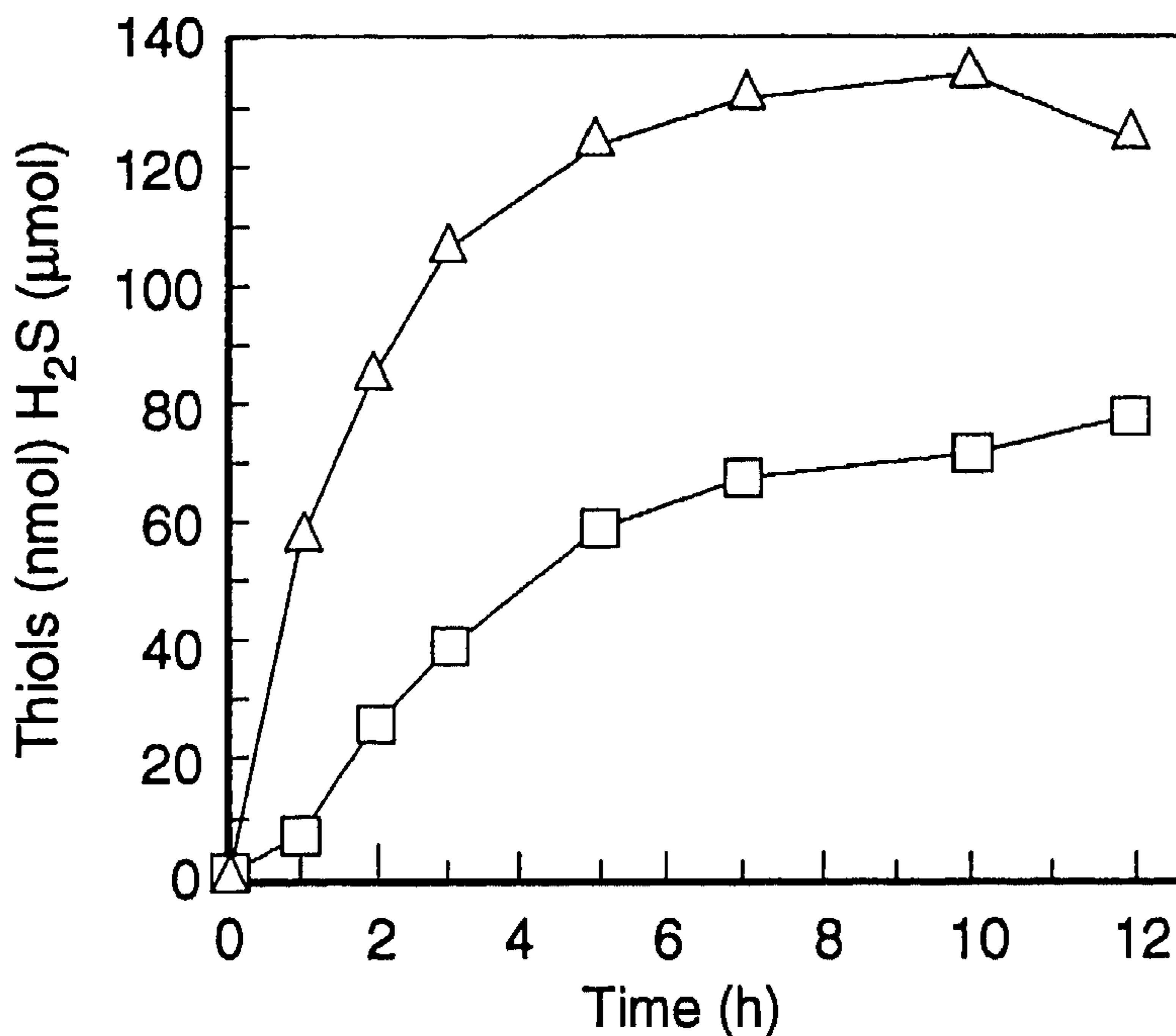


Figure 5. Generation of H₂S (triangles) and thiols (squares) with 0.855 mmol FeS and 1.0 mmol HCl and 90 °C during the first 12 hours. (H₂ formation stayed below 2 μmol and is not given in the graph).

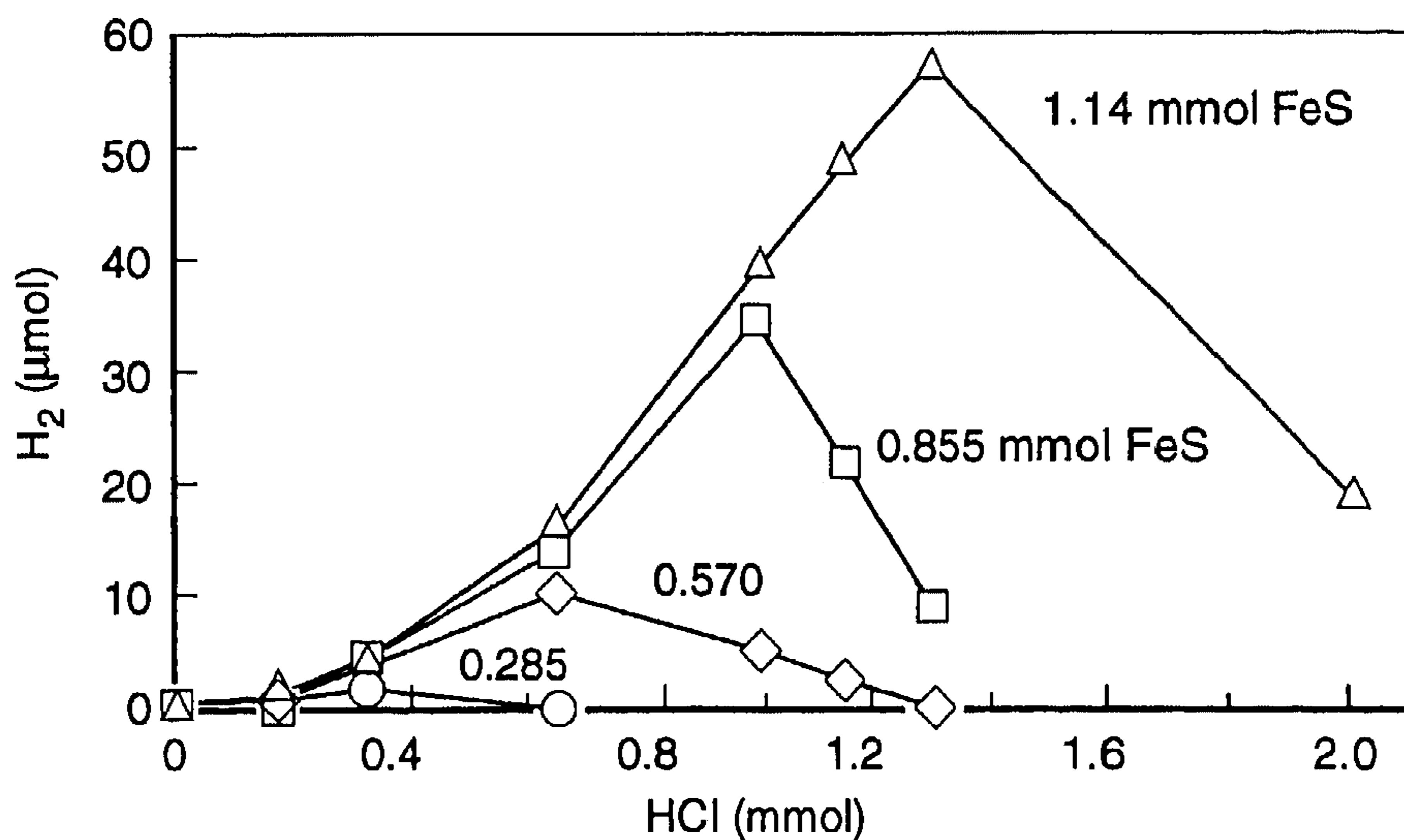


Figure 6a.

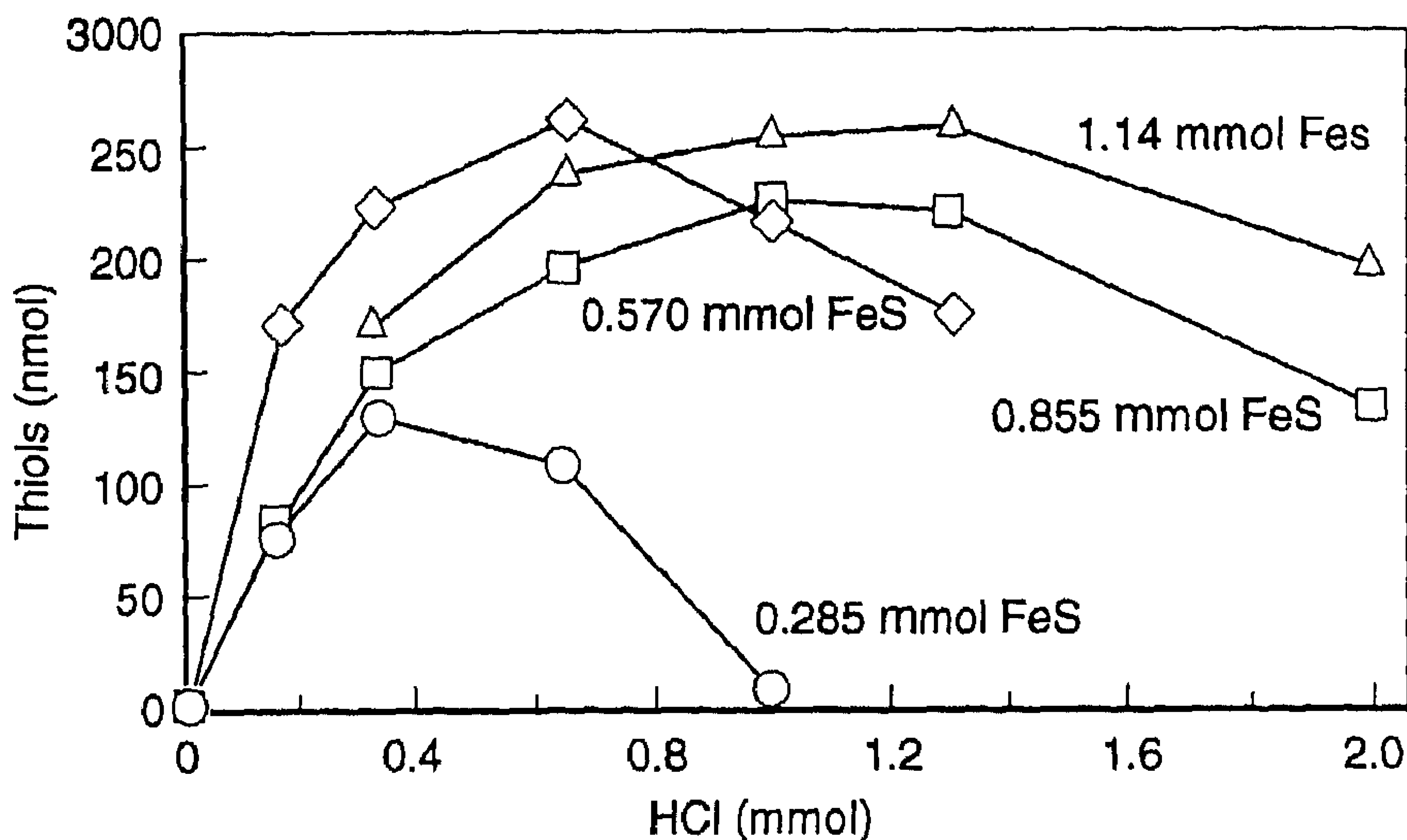


Figure 6(a)–(b). Formation of H_2 (a) and thiols (b) with 0.285 (circles), 0.570 (diamonds), 0.855 (squares) and 1.14 (triangles) mmol FeS and various amounts of HCl after (respectively) 3, 5, 7, or 10 days at 90 °C.

these data we conclude, that optimal thiol synthesis is ensured, if there is 15% more HCl than FeS. The duration of the reaction time increased with the amount of FeS and can be defined as approximately the given amount of FeS in mg divided by ten, with a margin of 1/10 (e.g. 0.57 mmol FeS = 50 mg FeS = 5 ± 0.5 days, at 90 °C).

The range of optimal conditions for H_2 and thiol formation were found to be quite different: The optima for H_2 production were quite sharp (Figure 6a), those for thiol synthesis in contrast very broad (Figure 6b). Alterations of the FeS- and HCl concentrations had a crucially different effect on H_2 and thiol generation: while H_2 formation increased significantly, the yield of thiols was hardly altered. The observed increase of H_2 without a corresponding increase of thiols, upon doubling of the quantities of FeS and HCl, was found to be due to the limitation of CO_2 in the N_2/CO_2 (80:20) gas mixture routinely used. When this mixture was replaced by a 100% CO_2 gas phase, thiol production was found to increase significantly (Table III) and was most pronounced at the optimum FeS/HCl-ratios.

The experiments demonstrated that optimum conditions for thiol synthesis are determined by a particular combination of reaction time and FeS/HCl ratio, and a sufficient supply of CO_2 , which changes with the amounts of FeS and HCl present in the system. With up to 0.57 mmol FeS with 0.65 mmol HCl, maximum yields of thiols could be obtained with the 80:20 N_2/CO_2 gas phase, but beyond 0.85 mmol FeS (with the corresponding amount of HCl) the 100% CO_2 gas phase was necessary to achieve optimum conditions for thiol synthesis (Figure 7). Because

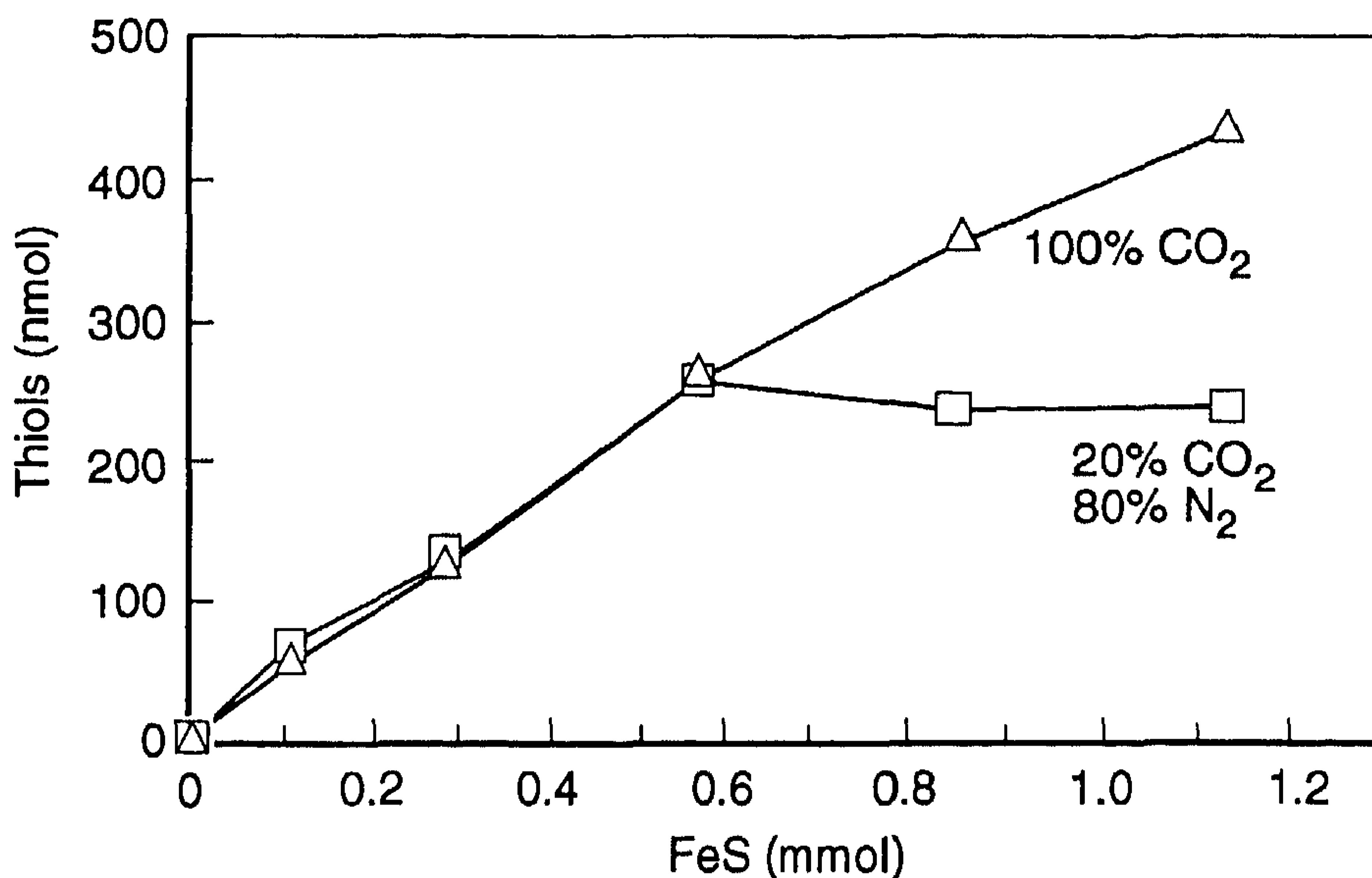


Figure 7. Thiol synthesis with 0.114, 0.285, 0.570, 0.855 and 1.140 mmol FeS with the corresponding amounts of HCl, after 1, 3, 5, 7 or 10 days respectively, with (squares) a 20% CO₂ (N₂/CO₂) gas phase, or (triangles) a 100% CO₂ atmosphere, all at 90 °C.

CO₂-deficiency has this restrictive effect all further experiments were carried out with a 100% CO₂ gas phase.

The reaction temperature was found to be another major parameter controlling the reaction sequence (Figure 8a and b), especially the formation of thiols and the generation of H₂ (Figure 8a). The production of H₂ which was negligible below 25 °C, increased slowly from 25 °C onward, then increased linearly with temperature between 50 and 75 °C, and finally decreased beyond that temperature. Thiol synthesis was found to proceed almost linearly but slowly up to 50 °C, then faster between 50 and 75 °C, and faster beyond 75 °C. These data show, that both reactions are temperature dependent, but not at the same level: The coincidence of the reversal of H₂ accumulation and the beginning of the accelerated thiol synthesis at 75 °C suggest, that at this point thiol formation is approaching its optimum temperature and thus consumes increasing amounts of H₂ for CO₂ reduction. We thus assume, that the optimum temperature range for H₂ generation is lower than the one for thiol synthesis.

Determinations of the primary reaction, the generation of H₂S from FeS and HCl (Figure 8b), confirmed that H₂S-formation is scarcely temperature-dependent, but the consumption of H₂S for H₂ production and thiol synthesis accelerated with increasing temperatures. Two other observations should be noticed in this context: At temperatures below 25 °C, thiol formation never proceeded for more than 7 days at low level, after this it collapsed totally. Simultaneously the proportion of

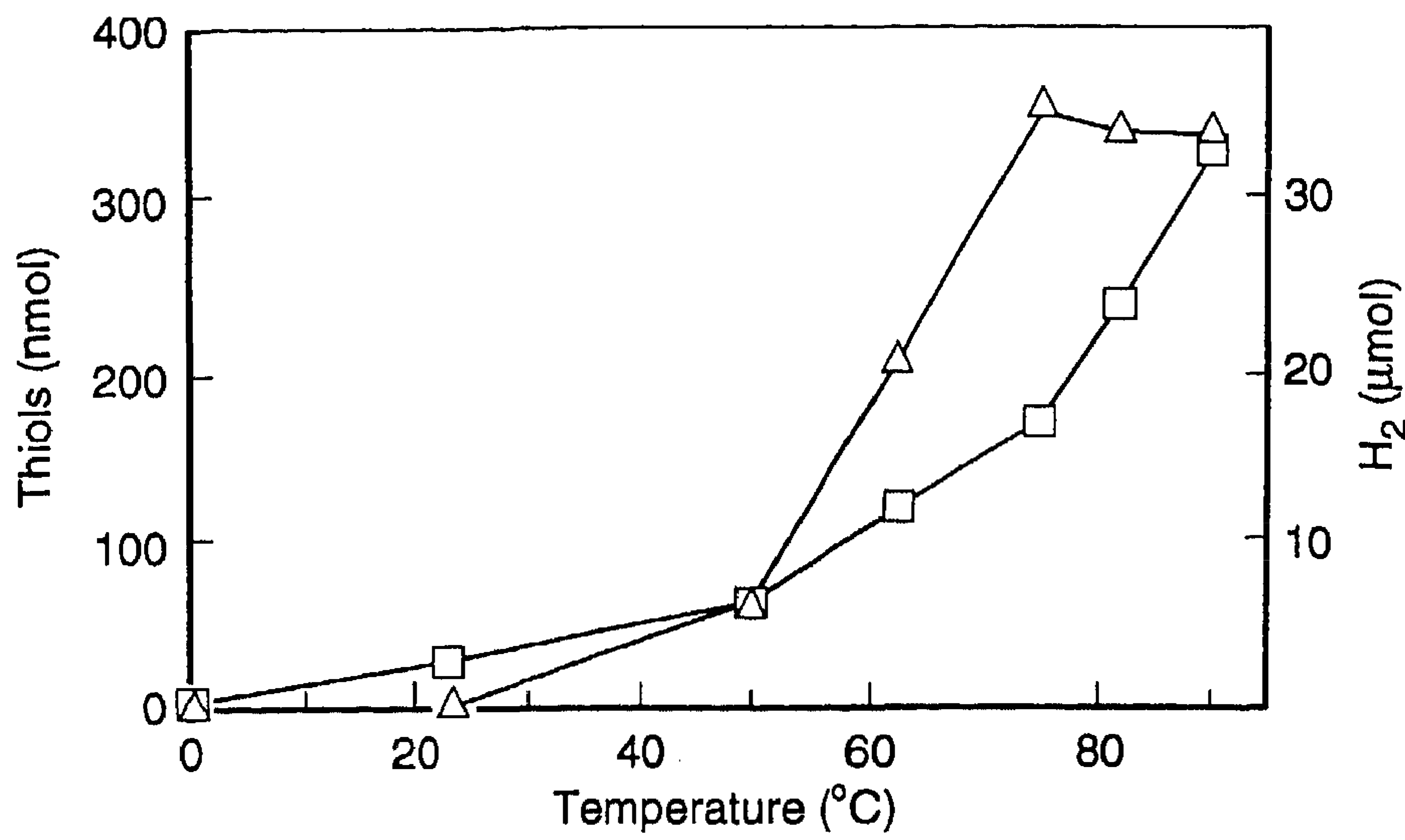


Figure 8a.

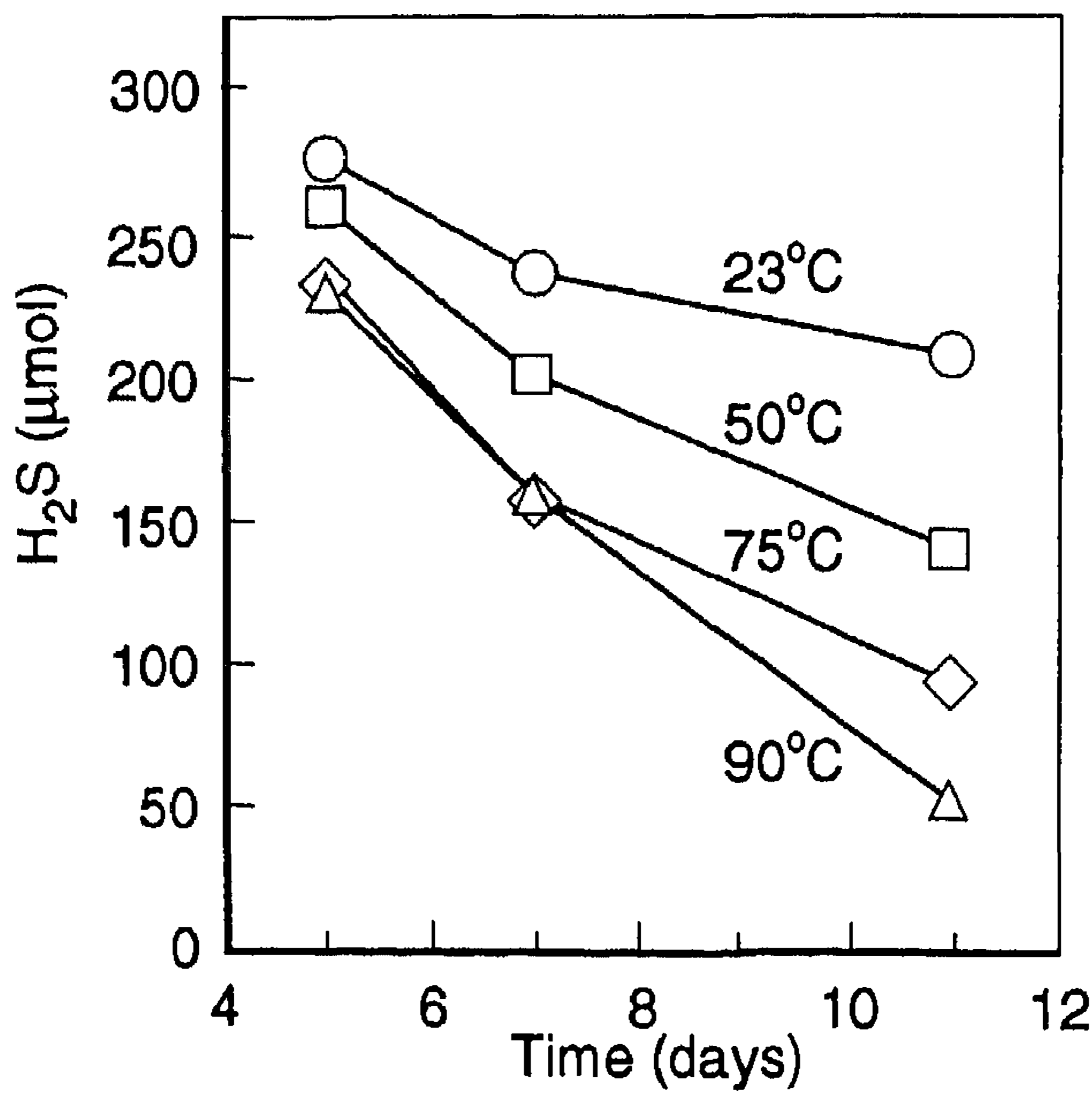


Figure 8(a)–(b). (a) Effect of temperature on the formation of H₂ (triangles) and the synthesis of thiols (squares), with 0.855 mmol FeS and 1.0 mmol HCl after 6 days. Note the different scales for H₂ and thiols. (b) Formation and consumption of H₂S under the same conditions at 23 °C (circles), 50 °C (squares), 75 °C (diamonds) and 90 °C (triangles).

Table IV

Controls with 570 μ mol FeS and variations of gas phase, additions and reaction time. Entries 1 and 2 represent standard conditions for the FeS/H₂S/CO₂- and the FeS/HCl/CO₂-system.

No.	Time at 90 °C	Addition	Gas phase	Thiols (nmol)	CS ₂ (nmol)	%	H ₂ S (μ mol)	Mineral product
1	5 days	150 μ mol H ₂ S	100% CO ₂	128.7	26.1	16.9	66.7	FeS ₂
2	5 days	0.65 mmol HCl	100% CO ₂	270.9	5.5	1.9	42.8	FeS ₂
3	30 min	0.65 mmol HCl	100% CO ₂	2.6	—		75.4	none
4	5 days	none	100% CO ₂	0	50.4	100.0	17.6	FeO
5	30 min	none	100% CO ₂	0	0	—	0.22	none
6	5 days	0.65 mmol DCl ^a	100% N ₂	0.5 ^b	4.4	89.6	155.5	(FeO)
7	dry heating at 90–120 °C			0	0	—	0	—

^a Water phase replaced by D₂O.

^b Methanethiol only, but at the detection limit.

CS₂ increased drastically (see Table II) and often represented the main product of the reactions.

Several controls were done to check for possible sources of contamination (Table IV). Comparison of the standard and a very short reaction time (entries 2 and 3, see also Figure 4) showed that H₂S production starts immediately, while there is little thiol formation. If neither H₂S nor HCl were added (No 4), some H₂S was formed and FeO was produced as mineral product (according to $\text{FeS} + \text{H}_2\text{O} = \text{FeO} + \text{H}_2\text{S}$). The amount of H₂S produced was not sufficient to get thiol synthesis started, but produced only CS₂. The FeO appeared (also in No. 6) as a small ring on the glass surface at the liquid/gas interphase. After a very short reaction time, without additions (No. 5), H₂S was the only product detectable, suggesting that traces are present from start. Finally, the 100% CO₂ gas phase of the FeS/HCl-system was replaced by 100% N₂. DCl and D₂O from a sealed vial/bottle were used to avoid any CO₂-contamination of HCl and H₂O stored at usual laboratory conditions. The trace of CO₂ which nevertheless got in by handling, was entirely converted to CS₂ due to the high amounts of H₂S (D₂S) present, while thiols were actually below detection limit. In a further approach, dry FeS was heated to 90–120 °C for 3 to 72 h (entry 7), and samples for GC were taken at the given temperature, but neither thiols nor CS₂ could be detected. These results exclude a contamination with thiols, because we have observed that monolayers of low molecular weight thiols, bound to a mineral surface, are released from the surface into the gas phase already at much lower temperatures (unpublished data).

Since the FeS from the different sources are all not 100% pure, small amounts of metallic iron could be present and cause H₂ production. We have done many experiments with Fe⁰ (unpublished data), which showed that H₂ formation increases linearly with the amount of metallic iron. Based on these data, 100 mg FeS (1.14 mmol) should yield 3 μ mol H₂ if the 0.1% impurity would be entirely Fe⁰. For

Table V

Identification of methanethiol and ethanethiol by mass spectroscopy. The main peak of each run was set as 100%. Row "H" = standard conditions, "D" = reaction system with D₂O and DCl. According to the data base used for comparison, methanethiol appears as mass 47 and 48 with a ratio of 100.0 : 90.3, and ethanethiol as mass 61 and 62 with a 13.6 : 100.0 ratio.

Methanethiol			Ethanethiol (set as 100%)					
Mass	% Base		Mass	% Base		Mass	% Base	
(MW)	"H"	"D"	(MW)	"H"	"D"	(MW)	"H"	"D"
47	100.00	10.21	61	18.98	1.40	82	0.27	0.17
48	80.48	38.74	62	100.00	7.51	83	—	0.93
50	2.90	100.00	64	9.74	17.82	84	0.12	0.87
51	1.60	55.66	66	0.62	36.54	89	1.23	0.31
52	—	51.65	67	0.38	100.00	90	12.18	0.11
53	0.10	8.11	68	0.37	93.19	92	—	0.31
54	0.80	3.00	69	0.20	10.81	98	0.10	0.81
56	0.70	0.62	76 ^a	14.03	15.27	99	—	6.22
58	1.30	0.90	76 ^b	3.72	0.18	102	—	6.31
60	0.14	0.51	78	0.22	0.18	102	—	0.40

controls, excess amounts of the iron sulfides of up to 5.7 mmol in H₂O under 100% CO₂ at 90 °C were used, but formation of H₂ could not be detected. Again, FeO was the only mineral end product.

Concerning the amounts of thiols given in this paper, one should realize that they represent only the thiols present in the gas phase at room temperature, whereas the amount in the liquid phase is not included in these data. The distribution coefficient has been experimentally determined as being 10.0 for methanethiol (Dr. A. Pol, pers. comm.), which is very close to the 9.88 given in the literature (Przyjazni *et al.*, 1983). Based on these values, 400 nmol thiols in 50 ml headspace give 800 nmol in 10 ml H₂O, thus a total of 1200 nmol.

To confirm the gas chromatographic identification of the sulfur compounds, samples from a 10 days reaction with 1.14 mmol FeS and 1.3 mmol HCl were analysed by mass spectrometry. Under normal conditions (Table V, left and right part row "H") methanethiol was found to be present as CH₃S[−] (mass 47) and CH₃SH (48) in a 1.0 : 0.8 ratio, while ethanethiol was solely present as C₂H₅SH (62). The compound with mass 76 and a retention time of 3.1 minutes (76^a) was identified as CS₂, whereas mass 76 with a retention time of 5.3 min (76^b = propanethiol) was present in too low amounts to give reliable data. Butanethiol (90) was found to be present as C₄H₉SH only.

Mass spectrometry was further used to demonstrate that the thiols are synthesized from the inorganic starting compounds. For that purpose, experiments under the same conditions as above, were carried out with D₂O instead of H₂O and DCl replacing HCl. The shift to the corresponding deuterium compounds, due to the

Table VI
Comparison of normal ("H") and deuterated ("D")
methanethiol and ethanethiol (and fragments) by
mass spectroscopy

Compound	"H"		"D"	
	Mass	(%)	Mass	(%)
CH ₃ S ⁻	47	100.0		
CH ₃ SH	48	80.5		
CD ₃ S ⁻			50	100.0
CD ₃ SH			51	55.70
CD ₃ SD			52	51.7
C ₂ H ₅ S ⁻	61	19.0		
C ₂ H ₅ SH	62	100.0		
C ₂ D ₅ S ⁻			66	36.5
C ₂ D ₅ SH			67	100.0
C ₂ D ₅ SD			68	93.2

incorporation of deuterium from DCl, the sole source for the reduction of CO₂ and the resulting formation of thiols (see equations (1-4)) is given in Table V (left and right, row "D"). Methanethiol, present as CH₃S⁻ and CH₃SH appeared in its deuterated form (Table VI) also as CD₃S⁻ (mass 50) and CD₃SD (52). Almost the same amount is present as CD₃SH (mass 51), due to the very high affinity of the sulfur group to hydrogen, a clear preference for S-H. Consequently, the deuterium of the SD-group is exchanged for hydrogen if even the slightest trace is available, be it as H₂O of hydration or H₂S in FeS, or simply H₂O introduced by handling. The same holds for ethanethiol, with almost even amounts of C₂D₅SH (mass 67) and C₂D₅SD (68), whereas C₂D₅S⁻ (66, 36.5%) is present in somewhat higher amounts than the corresponding C₂H₅S⁻ (61) under standard conditions (19.0%). The low amounts of mass 83 and 84 (C₃D₇SH and C₃D₇SD, Table V) were not reliable, but butanethiol was present as C₄D₉SH (99) and C₄D₉SD (100). These results reveal the incorporation of deuterium into the thiols, i.e. the reduction of CO₂ by the deuterium introduced with DCl (see equations (1-4). A control run with 1.14 mmol FeS in 10 ml D₂O, under the same conditions as before, but without H₂S or HCl (to prevent the formation of active H₂ and the synthesis of thiols), with approximately 1.4 μmol methanethiol (±350 nmol in the headspace), was carried out to determine, whether a deuterium exchange with the hydrogen of the methyl-group would occur. The GC/MS analyses gave no evidence for such a substitution. The only peaks for methanethiol present were mass 47 and 48.

A comparison of the FeS/H₂S/CO₂- and FeS/HCl/CO₂-system together with several controls is given in Table VII. Entries No. 1 and 2 confirm, that negligible traces of CS₂ and DMDS originate from impurities of (the 99.9% pure) FeS, and that thiols are totally absent. The following entries (No. 3–5) represent the

Table VII

Formation of organic sulfur compounds (nmol thiols, CS₂ and DMDS) from variations and combinations of FeS, H₂S, H₂ and HCl (100% CO₂ gas phase, 90 °C).

No.	FeS ^a	H ₂ ^b	mmol 1.0	HCl 0.13	H ₂ S ^c	Total (all values = nmol)				Percent value ^d
						OSC	thiols	CS ₂	DMDS	
1	+	–	–	–	–	1.0	0	0.5	0.5	0
2	+	+	–	–	–	1.2	0	0.8	0.4	0
3	+	–	+	–	–	59.7	30.5	26.1	3.1	42.5
4	+	+	+	–	–	64.3	32.9	30.4	1.0	45.8
5	+	–	–	+	–	81.7	71.0	7.0	3.8	98.9
6	+	–	–	–	+	84.4	71.8	10.2	2.5	100.0
7	–	+	–	+	+	33.2	15.5	17.2	0.6	21.6
8	–	+	–	–	+	30.1	19.6	10.1	0.4	27.4

^a 114 μ mol.

^b 240 μ mol.

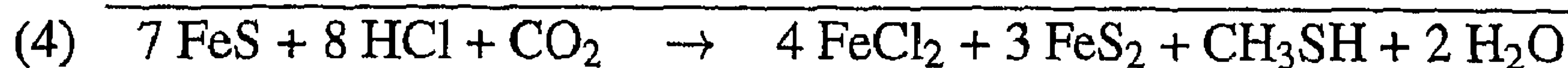
^c 120 μ mol.

^d Thiol formation of all combinations related to no. 6 (FeS + H₂S) as 100%.

FeS/HCl/CO₂-system. Comparison of No. 3 and 4 reveal that additional H₂ had no effect on thiol synthesis. Comparison of entries No. 3 and 5 again emphasize the importance of a particular proportion of FeS and HCl: with an excess of HCl (No. 3) thiol formation was relatively low and partially replaced by production of CS₂. The last two entries (No. 7 and 8) show that these compounds can also be formed from H₂, H₂S and CO₂ in the absence of FeS, both under acid and neutral conditions, although with lower yields. The lower thiol formation in acidic environment was again due to enhanced CS₂ production (51.6% in No. 7 vs. 33.7% in No. 8). The data show, that thiols can be formed under the given conditions (which include a sufficient supply with CO₂) in three ways: From FeS and HCl, generating primarily H₂S and secondarily H₂, from FeS and H₂S with the generation of H₂, and from H₂ and H₂S; the latter is, however, much less effective and produces relatively high quantities of CS₂.

4. Discussion

In summarizing the results, we propose the following scheme for the formation of thiols *via* the FeS/HCl/CO₂- and the FeS/H₂S/CO₂-system (which starts with reaction (2)). We thereby suggest an experimentally corroborated alternative to the Miller electric discharge reactions (Miller, 1953, 1955, 1957).



Several conclusions can be reached from the experimental results and these equations:

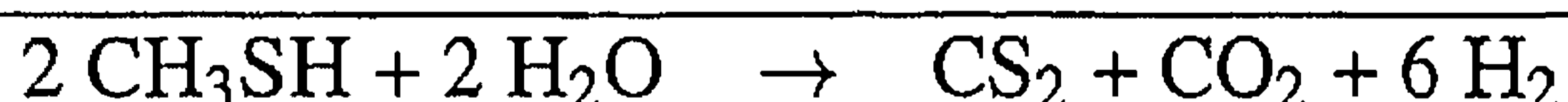
(1) Reaction (1) shows little time and temperature dependence, but varies significantly with the relative abundances of FeS and HCl.

(2) Reaction (2) depends on temperature and accelerates at 50 °C.

(3) With the FeS/HCl/CO₂-system reactions (1) and (2) are strongly influenced by the FeS/HCl ratio.

(4) The latter holds also for reaction (3), which, however, is significantly affected by both time (Figure 4) and temperature (Figure 8), and it depends on having a sufficient supply with CO₂. This reaction can proceed independently of the two others, in absence of FeS, however with quite high yields of CS₂ as byproduct.

(5) Whether thiols or CS₂ (and other S-compounds) are the main products of the reaction sequences, depends mainly on the proportion of FeS and HCl, due to the competition of reaction (1) and (2) for FeS. With high amounts of FeS and low amounts of HCl (and *vice versa*) the bulk of the FeS is consumed according to reaction (1), yielding high quantities of H₂S, and leaving insufficient amounts of FeS for the second reaction and thus a deficit of H₂. Assuming COS as an intermediate (reaction 5), an excessive production of H₂S accompanied by a scarcity of H₂ would interfere with the formation of thiols under favorable conditions (reaction 6), and render instead CS₂ as the main product (reaction 7 and 8). Reaction (8) is confirmed by experimental evidence: With low quantities of H₂ available, we have frequently observed the appearance of low amounts of thiols, which disappeared when the H₂ was consumed; instead of thiols we then obtained CS₂ as the sole product. Furthermore it was observed that methanethiol, in an aqueous environment at 90 °C without H₂, decreased with time, while small amounts of COS, and increasing quantities of CS₂ appeared. Increasing yields of CS₂ are always indicative of suboptimal conditions for CO₂ reduction (thiol formation): a limitation of H₂, resulting either from an unfavorable FeS/HCl (H₂S) ratio or a low reaction temperature. A further indicator of unsatisfactory conditions is the occurrence of DMDS in more than trace amounts: it is presumably produced by condensation of two molecules methanethiol, if the formation of longer thiols cannot be achieved due to the lack of H₂.



(6) The occurrence of COS as an intermediate, as postulated in reaction (5), can so far not be demonstrated quantitatively, because only under certain conditions,

when the H_2S peak did not interfere, was it possible to identify and determine the COS-peak. These few data, however, are not sufficient to render any conclusions on the changing quantities of this compound.

(7) Depending on the amounts of FeS and HCl present in the system, the availability of CO_2 can become a decisive parameter: Above 0.85 mmol FeS (and the corresponding amount of HCl) an 80:20 N_2/CO_2 gas phase is not sufficient to guarantee maximum yields of thiols and has to be replaced by a 100% CO_2 atmosphere. The CO_2 supply is, however, not a real problem, because it arises only with our experimental (= closed !) system; in an open system it would be of no relevance.

(8) The reaction product FeS_2 appears – at least in part – as a silvery floating layer on the aqueous surface, most probably because a coating with thiols renders a hydrophobic surface.

(9) The deuterium experiments demonstrate the genuine reduction of CO_2 (Tables V and VI) by the reductants D_2S and D_2 . In this case, the primary hydrogen source HCl has to be replaced in reaction (1) by DCl .

(10) The organic sulfur compounds are presumably neither the only, nor the final products, if such reactions occurred in hydrothermal systems where “numerous metastable equilibria allow for a wide variety of chemical species to coexist” (Hennet *et al.*, 1992; Shock, 1992), but could rather serve as precursors for the synthesis of non-sulfur organic compounds, e.g. amino acids (Hennet *et al.*, 1992, Yanagawa and Kobayashi, 1989; Russell *et al.*, 1994). Furthermore one of the participants required to open the way to the thioester world (de Duve, 1991, 1992) becomes abundantly available by the appearance of thiols on the prebiotic stage.

(11) A reducing atmosphere, a prerequisite for the emergence of the primordial soup *via* electric discharge reactions, is not required for the synthesis of thiols by both systems, since they could just as well be formed in a CO_2+CO dominated atmosphere, or a CO_2+N_2 atmosphere containing traces of CO , H_2 and reduced sulfur gases, during or after the “heavy bombardment” period (Kasting, 1993). Then as now the system could operate in submarine hydrothermal vents, either in presence of FeS , H_2S and CO_2 , or (in absence of H_2S) with FeS and CO_2 alone in a moderate acid environment (Macleod *et al.*, 1994; Shock *et al.*, 1995).

(12) Wächtershäuser has postulated the formation of carbonic acids as primary products of the pyrite-driven reaction. This could be the case in the reaction of H_2 with FeS (entry 2, Table VII), but we have not looked for non-sulfur compounds. But entry 5 of Table VII shows, that organic sulfur compounds are the primary products, if even the slightest acidity is given in the environment. A modification of Wächtershäuser’s proposals in that respect, that the primary products of the pyrite-pulled reaction are organic sulfur compounds, could probably pave the way to de Duve’s thioester world unfolding on a mineral surface.

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