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Determination of molecular sulphur in sodium thiosulphate injection solutions by high performance liquid chromatography

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A method was developed to quantify the sulphur formed in decomposed sodium thiosulphate injection solutions. Elemental sulphur was extracted with cyclohexane (LLE) and analysed by RP-HPLC. A linear calibration curve with a detection limit of 24 μg/ml was obtained. Different amounts of sulphur were detected in extemporaneous and commercial solutions.

1. Introduction

Sodium thiosulphate is used as an antidote in cases of intoxication e.g. with cyanide, cyanogenic compounds, lost, mustard, or nitroprusside and to prevent toxic effects of cisplatin [1–7]. The usual preparations are solutions for injection with concentrations of 10% or 15% sodium thiosulphate pentahydrate. The concentration of commercial infusion solutions is normally 25% sodium thiosulphate pentahydrate. The compound is standardised in the European Pharmacopoeia [8]. The USP includes an extra monograph with standards for the injection solution [9]. The decomposition of aqueous sodium thiosulphate solutions is a known phenomenon, but the degradation process is still not completely understood [10, 11]. Solutions become cloudy and precipitation of sulphur occurs. The pH value of the solution is important for the stabilisation of a pharmaceutical preparation [9–13]. Acidified solutions have a great tendency to degradation.

The decomposition of highly concentrated thiosulphate injection solutions has been examined only infrequently. Possible degradation products of thiosulphate injections are sulphate, sulphite, sulphur, polythionates, sulphide, and polysulphides. Hitherto only sulphate, sulphite, sulphide, and sulphur have been reported as having been identified by some authors [12–14]. Other compounds were not found. The decomposition of thiosulphate has been described in a considerable number of publications [15]. The described reactions formed are diverse, very complex, and influenced by a number of parameters. The resulting compounds are those listed above. Under certain conditions tetrathionate is also detectable. Polythionates with longer chain length and polysulphides occur in addition to sulphate, sulphite, and different sulphur-homocycles under extreme conditions. Sulphide, sulphite and sulphur may be detected if a thiosulphate solution is acidified. Sulphate occurs in alkaline conditions or by oxidation of sulphite, sulphide, or polythionates. Heavy metals influence the route of decomposition by their catalytic action [15–26]. In order to obtain stable preparations, formulations without disulphite and ethylenediaminetetraacetate [12] or heating and filtration of the batch before filling [13] have been recommended.

This work directs its attention to the determination of molecular sulphur. Sulphur is described as being insoluble in water and poorly soluble in organic solvents except carbon disulphide [27]. The extraction of molecular sulphur from an aqueous medium is normally done with carbon disulphide. A published method to determine sulphur in thiosulphate injection solutions describes extraction of the sulphur with carbon disulphide. The extraction is followed by evaporation of the solvent. A final reaction with sodium nitroprusside results in a violet coloured complex. This method was designed only as a limit test, and no calibration was made. A polarographic or ionchromatographic determination was not practicable due to the small amount of sulphur in contrast to the high thiosulphate concentration [12].

A range of sulphur homocycles and polythionates has been determined by Steudel et al. in sulphur hydrosols [28, 29]. The investigation was made after extraction with carbon disulphide or with cyclohexane from so-called Raffo Sols and Selmi Sols. The extracts were analysed by RP-HPLC. Raffo Sol can be obtained by acid decomposition of thiosulphate. Selmi Sol can be obtained by reaction of aqueous sulphide and sulphite at low pH value. The hydrophilic character of the sols is important. It is caused by the incorporation of molecular sulphur in micelles formed by polythionates. During an ageing process of Raffo Sol a shift in the composition has been observed. The concentration of stable sulphur compounds and polythionates with small chain length increased [28].

2. Investigations and results

In order to prove the robustness of the method, examination of different thiosulphate injection solutions was planned. Therefore both commercial products and extemporaneous solutions were investigated. The extemporaneous solutions were prepared such that different kinds of decomposition products could be expected. The pH value, and the addition of buffer system, antioxidant, and heavy metal chelating agent were varied. Every formulation was prepared three times in the same manner with an interval of 12 months. For the second batch the bulk solution was divided into two parts (except formulation IV – all 3 batches were divided). One half was filtered through a filter material with a pore size of 0.20 μm and filled in ampoules. The other half of the bulk solution was filtered, filled in ampoules and steam sterilised (Table).

The ampoules were stored in darkness and at ambient temperature. Most of the solutions showed symptoms of decomposition after a few months. They became cloudy and small almost white needles or crystalline particles were formed as a precipitate. It is obvious that this precipitate is molecular sulphur in the most stable S_8-form. The formation of a hydrosol with stabilising polythionate micelles under this conditions is improbable. The small amount of precipitate requires quantification in the ppm range. The aim of this paper is to examine if liquid-liquid extraction and determination of sulphur formed in thiosulphate injection solutions is possible.

First a suitable HPLC method was developed and tested. The reversed phase technique with a mobile phase com-

Table: Formulations of sodium thiosulphate injection solutions

Bulk number or commercial preparation	$Na_2S_2O_3 \cdot 5\ H_2O$ (declared content)/g	$Na_2S_2O_5$/g	EDTA/g	NaOH/g	KH_2PO_4/g	Na_2HPO_4/g	Aqua ad injectionem/g	Content/%	pH Adjustment (average)	pH After sterilisation (average)
I	100.0	–	–	–	–	–	ad 1000.0	10	no adj. (meas. ~7)	5.3
II	100.0	0.100	1.000	–	0.050	1.120	ad 1000.0	10	no adj. (meas. ~7)	6.5
II	100.0	0.100	–	q.s.	–	–	ad 1000.0	10	adj. to ~8.5	7.4
IV	100.0	–	–	0.014	–	3.850	ad 1000.0	10	adj. to ~9.0	8.2
V	250.0	–	–	0.034	–	10.010	ad 1000.0	25	adj. to 9.4	8.9
IX	100.0	–	–	0.034	–	10.010	ad 1000.0	10	adj. to 9.4	9.0
X	250.0	–	–	0.034	–	10.010	ad 1000.0	25	adj. to 9.4	8.9
Köhler Ch. 961911	100.0	0.100	+*	+*	–	+*	ad 1000.0**	10	not known	not known (meas. ~ 8.9.)
Anfarm Lot1201/04/2002	250.0	–	–	–	–	–	ad 1000.0	25	not known	not known (meas. ~7.0)
Apoteksbolaget Lot 704X2372	150.0	–	–	–	–	6.00	ad 1000.0	15	not known	8.2–8.8 (decl.) (meas. ~8.3)

* declared as additive without speciying of quantity
** glycine and sodium chloride are declared as further additives without specifying of quantity

posed of methanol and cyclohexane was chosen. This approach takes into account the results of Steudel et al., the nonhydrophilic character of the sulphur in the ampoules, the low UV absorbance of methanol, and the evident sulphur solvating ability of cyclohexane. In order to determine the retention time of sulphur a number of pure sulphur solutions were measured first. The linear calibration range and the signal response of different sulphur concentrations ion online UV detection were examined. For this an appropriately diluted stock solution of sulphur in cyclohexane was used.

The measured retention time of sulphur was about 2.05 min. The capacity factor was $k' = 2.41$. Additional peaks occurred neither after injection of sulphur concentrations in the linear calibration range nor after sample overload. The quantity of 30% cyclohexane was varied in the range of $\pm 5\%$. With increasing cyclohexane the retention time of sulphur decreased about 0.13 min and vice versa. The capacity factors changed by ± 0.2. With the exception of the retention time no differences in the chromatograms were observed. The final solvent composition was methanol/cyclohexane (70/30). The peak obtained after the extraction of thiosulphate solution spiked with sulphur (500 µg/ml) was symmetrical regarding the symmetry factor $S_s = 1.0$. The peak obtained after the extraction of an injection solution (batch IV A) was symmetrical regarding the symmetry factor $S_s = 1.1$. The capacity factor was $k' = 2.31$. No peak shoulders were observed.

The determination was limited in the upper range by an excessive signal due to the high absorption coefficient of sulphur. Integrable signals were obtained below 1000 µg/ml sulphur in cyclohexane. A calibration curve obtained from the relationship of peak area versus concentration in the range between 100 µg/ml and 900 µg/ml was linear (Fig. 1). It can be described by the eq. $y = 9710.194\ x + 122897.38$. The correlation coefficient was about 0.9959. The standard deviation of the slope was 1.5%. The standard deviation of the intercept was 66% and therefore the intercept does not differ significantly from zero. The detection limit was about 24 µg/ml. The limit of quantification was about 108 µg/ml ($F = 19$, $\alpha = 0.05$, $k = 4$) (Fig 1). The relative standard deviation of the measured peak areas was about $\pm 1.2\%$. The average error in recovery of spiked standards was $\pm 1.4\%$. The standard deviation of the content was about ± 12.0 µg/ml.

In order to determine the sulphur formed, extraction from the aqueous injection solution is necessary. A calibration has to be done under extraction conditions because complete quantitative extraction cannot be assumed. Concentration dependence of the process is probable. All quantities were doubled in view of the range of the balance used to achieve a larger mass of sulphur to weigh out. The addition of the sulphur was deliberately not by a stock solution because the sulphur in the injections is not present as a dissolved substance either. Therefore different amounts of sulphur were added to the appropriate volume

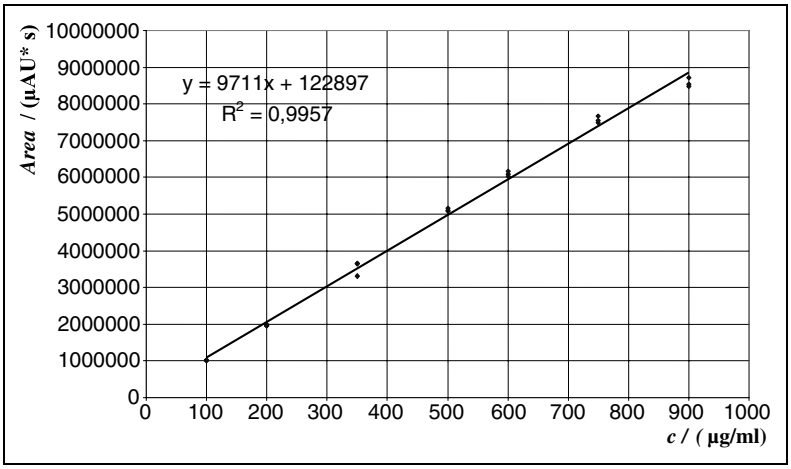

Fig. 1:
Calibration curve obtained by relationship of peak area versus concentration: different amounts of sulphur dissolved in cyclohexane analysed by HPLC

of thiosulphate solution. The thiosulphate concentration was identical to that of the injection solutions. It is important to transfer the precipitated sulphur completely. Therefore the volume of the rinse liquid has to be taken into account. The rinse volume for every ampoule was fixed at 10.0 ml of high purity water. An equivalent volume of high purity water was also added to the calibration solution. Cyclohexane was added as extraction medium to the total volume. The extraction was done by shaking on a waterbath. A small part of the organic phase was injected into the HPLC system.

First the required extraction time and the possible range of calibration were examined. 1.5 mg of sulphur in 10.0 ml of sodium thiosulphate solution was extracted for 3 h with 1.00 ml of cyclohexane. Injecting the organic phase a very large peak occurred and the detector range was exceeded. This result was compared with the data obtained from the sulphur solutions. The conclusion was that during 3 h more than 60% (c_S > 900 μg/ml) of the total amount of sulphur had been dissolved in the cyclohexane. In order to estimate the approximately required range of concentrations an injection solution with apparent sulphur precipitation was examined. A response in the upper third of the detector range occurred. The calibration range was sef between 200 μg/ml and 900 μg/ml sulphur in view of these results. The extraction time was shortened to 2 h. All other parameters were as described before. The ampoules were examined using an equivalent extraction procedure. Additionally a determination was performed on freshly prepared thiosulphate solution from the bulk material used for the extemporaneous preparations. From the result the initial sulphur content of the injection solutions made by ourselves was calculated.

A linear calibration curve was obtained by calculating the relationship of peak area versus concentration (Fig. 2). It can be described by eq. $y = 11906.72\,x - 1670301.5$. The correlation coefficient was 0.9851. The resulting detection limit was 59 μg/ml. The resulting limit of quantification was 189 μg/ml ($F = 10$, $\alpha = 0.05$, $k = 3$). The average error of recovery was ±3.7%. The relative standard deviation was about ±1.4%. The standard deviation of the content was about ±28.9 μg/ml.

The measured sulphur concentrations of the ampoules ranged from 296 μg/ml to 853 μg/ml sulphur. All calculated concentrations were above the limit of quantification. Some measured peak areas resulted in a concentration above the limit of registration ("Erfassungsgrenze DIN 32645") but below the limit of quantification. No value was beneath the limit of detection. For one batch dilution of the cyclohexane was necessary because of the high sulphur concentration after extraction. Using the eq. above for the calculation the obtained values (μg/ml) are equivalent to the concentration of sulphur in 1 ml cyclohexane. This value is equivalent to the concentration of sulphur in one ampoule (μg/10 ml), except when dilution was necessary. The highest concentration determined taking the dilution into account was 1387 μg sulphur in 10 ml injection solution. The initial concentration of sulphur of the thiosulphate was determined as 146 μg/g (±29 μg/g). The value was calculated as sulphur in sodium thiosulphate pentahydrate. This was equivalent to a sulphur concentration of ≈0.015%. The thiosulphate concentration of the injection solution in each case was determined in a separate investigation by differential pulse polarography [30]. Considering the thiosulphate concentration of each ampoules the sulphur concentrations ranged from 102 μg/g to 1249 μg/g (±29 μg/g) (Fig. 3). The values were calculated as sulphur in sodium thiosulphate pentahydrate. Determination of the sulphur formed by decomposition during storage was only possible for the extemporaneous injection solutions. The calculated concentrations of sulphur formed by decomposition ranged from 50 μg/g to 1118 μg/g (±29 μg/g). They were calculated as sulphur in sodium thiosulphate pentahydrate. For commercial preparations the total amount of sulphur was determined. The concentrations ranged from 81 μg/g to 123 μg/g (±29 μg/g) calculated as sulphur in sodium thiosulphate pentahydrate.

The amount of sulphur formed in the solutions correlated with the type of stabilisation. The highest quantities of sulphur were measurable in the injection solution without additives (bulk I). The ampoules had a pH of about 5.3 after sterilisation, and a final pH of about 4.9 after storage. The initial pH after preparation was about 7.0. The sulphur formed in the nonsterilised, filtered bulk was 1.5 times higher than in the sterilised bulk. In the cases of two bulks the sterilised batch had a higher degree of decomposition (bulk II and III). With the more stable alkaline ampoules (bulk IV) a difference was observed between the different batches. This bulk was examined over a period of 42 months divided into a filtered and a sterilised batch. The filtered batches (batch A) showed faster decomposition in the first 18 months and reached the final concentration early. No important change occurred over the two year period of further observation. The sulphur concentration in the sterilised ampoules (batch B) rose during storage. With the most stable ampoules (bulk IX) no significant sulphur formation was found. This bulk was

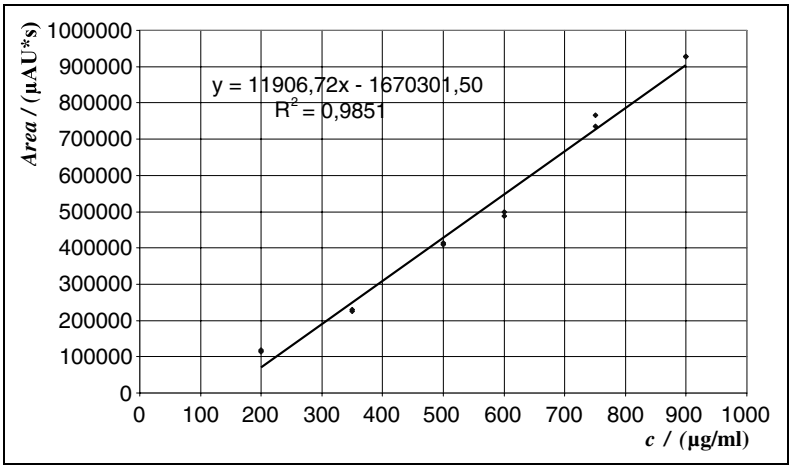

Fig. 2:
Calibration curve obtained by relationship of peak area versus concentration: cyclohexane extraction of spiked amounts of sulphur from 10 % sodium thiosulphate solution followed by HPLC analysis

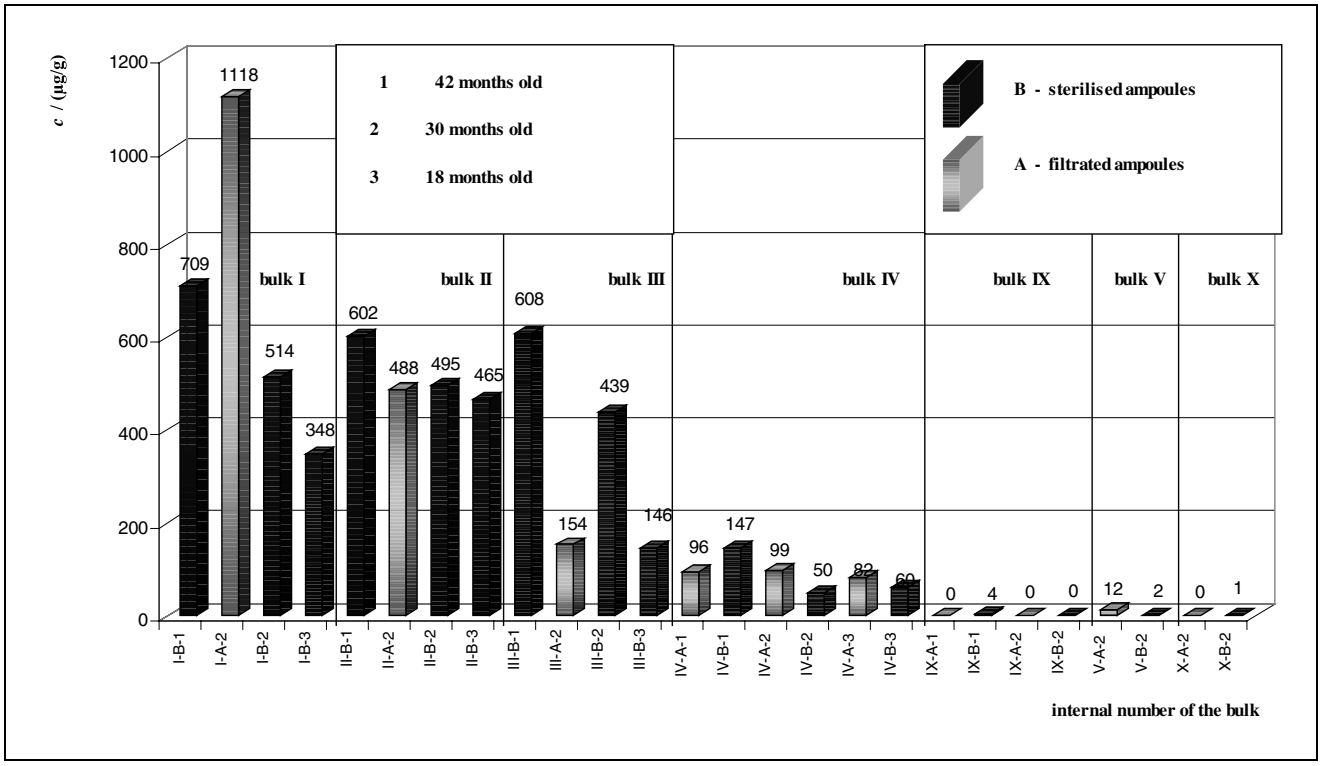

Fig. 3: Magnitudes of the measured sulphur concentrations formed by decomposition of extemporaneous thiosulphate injection solutions (relative to the concentration of sodium thiosulphate pentahydrate of each ampoule)

prepared with alkaline pH and a higher concentration of phosphate buffer system. In addition the whole batch material was filled into infusion vessels, steam sterilised and filtered before filling in ampoules. Then the batch was divided and the half batch was sterilised again (batch IX B). The values of sulphur concentration for formulations IV and IX are estimated because they ranged between the limit of registration ("Erfassungsgrenze DIN 32645") and the limit of quantification.

For the sterilised injection solutions examined over a period of 42 months the trend of a rising sulphur concentration during ageing was evident. The dependence of thiosulphate decomposition on the pH and the stabilising additives was found as expected. With a more alkaline pH up to 9.4 initially and a higher concentration of buffer additives the amount of sulphur formation tended to zero. Pre-sterilisation of the whole batch of a very alkaline and adequately buffered formulation (bulk IX) followed by filtration led to a very stable preparation. This suggests that a stable equilibrium state is reachable. Addition of ethylenediaminetetraacetate and sodium disulphite seemed to have no advantage if the pH was not stabilised in the alkaline range with an adequate buffer system. With the preparation with a combination of ethylenediaminetetraacetate and sodium disulphite additives, faster decomposition was observed in the first 18 months compared to the preparation with disulphite additive alone. The final sulphur concentration formed after 42 months was similar (602 µg/g bulk II; 608 µg/g bulk III). Both preparations had an insufficient buffer capacity. The final pH values were lowered from ≈7.0 to ≈6.5 (bulk II) and from 8.5 down to ≈7.4 (bulk III).

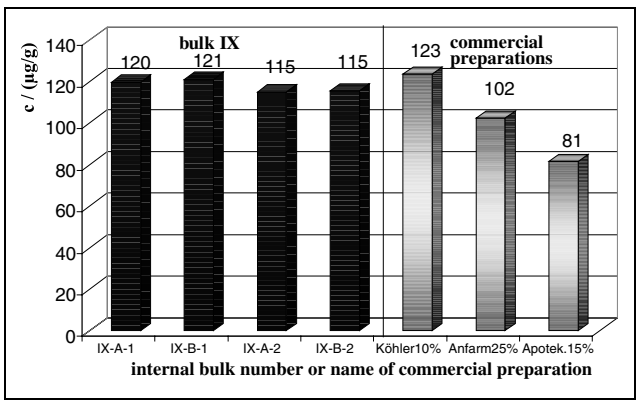

Fig. 4: Measured total sulphur concentration in commercial ampoules relative to the sodium thiosulphate pentahydrate concentration, compared to the batches of the sterilised bulk IX of extemporaneous solutions

Commercial solutions are adjusted to a high pH value of about 8.9 and prepared with an alkaline buffer system. In some cases the solutions are filled in ampoules made of brown glass. The addition of disulphite and ethylenediaminetetraacetate is common practice. Comparing a stable extemporaneous solution (bulk IX) to the stable commercial solutions no advantage was found. In both cases the amount of total sulphur determined was not significantly different (Fig. 4). Assuming the quality of the sodium thiosulphate used is comparable (equivalent sulphur concentration at the start) no sulfur formation had occurred during storage in either preparation.

In all the solutions investigated no other peaks were detected. The sulphur peak obtained was symmetrical and no peak shoulder was observed. Formation of other sulphur homocycles could not be shown.

3. Discussion

The method is suitable to determine the sulphur formed by decomposition of thiosulphate in aqueous solutions. It is shown that the extraction of crystalline, elemental, non-hydrophilic sulphur from an aqueous medium is possible

using a small amount of cyclohexane (in a ratio of five to one hundred). Furthermore quantification by HPLC analysis is practicable with sufficient precision and limit of quantification. The calibration range includes the magnitude of sulphur concentrations caused by degradation in high concentration injection solutions. The method is suitable for quality control of thiosulphate injection solutions.

The extraction time was chosen in order to use one method for the estimated lowest and the highest sulphur concentration. The wide concentration range of different decomposed solutions had to be taken into account. To detect smaller amounts of sulphur a longer extraction time could increase the potential of the method. If necessary, extraction at higher temperature, with a smaller amount of extraction solvent, or the inclusion of an evaporation step are possibilities. The method described has the advantage of making all determinations without the use of highly inflammable and toxic carbon disulphide. Extraction with cyclohexane is rapid. HPLC gives rapid determination with the precision of online detection. To obtain sufficient robustness and a high precisions exact temperature control, and identical geometry of the extraction flasks and shaking frequencies are necessary.

The most important factor for the stability of thiosulphate injection solutions is the pH value. The more alkaline the solution the more stable it is. A high buffer capacity is required to prevent a decrease of the pH value. The influence of other additives seems to be insignificant. A heating process and a filtration of sulphur formed before the final sterilisation lead to greater stability.

In this paper only S_8 was determined. It is expected to be the preferred existing form because it is the most stable allotrope. The fact that no other sulphur homocycles were found is no evidence that the species do not occur in such solutions. Trace amounts of homocycles as intermediates of the decomposition should be taken into consideration. In order to prove the existence of homocycles a method with high sensitivity and a very low detection limit is required.

4. Experimental

4.1. Preparation of the injection solutions

The injection solutions were prepared from sodium thiosulphate pentahydrate (AR > 99.5%, Riedel de Haën AG Seelze). The water was met quality requirements of the Pharmacopoeia for injection solutions (in-house circulating purified water system). Sodium hydroxide was used to adjust to an alkaline pH (sodium hydroxide AR, Chemapol Prague). The pH value was measured by potentiometry using a glass electrode (pH-Meter WTW pH 525, WTW; combined micro pH electrode [Ag/AgCl/3M KCl] N 6000 A, Schottgeräte GmbH Hofheim). For some preparations phosphate buffer was added (disodium hydrogenphosphate, DAB 7 DDR, potassium dihydrogenphosphate; 2. AB DDR; VEB Laborchemie Apolda). For special preparations sodium disulphite (>98% puriss., Riedel de Haën AG Seelze) and ethylenediaminetetraacetate (Caesar & Loretz GmbH Hilden) were used. For the formulation of each bulk solution see Table. The substances were dissolved in a special container and filtered through a filter with a pore size of 0.20 µm (Sartorius Type SM 17531 DJG Werkstoff, Satorius AG Göttingen; Celluloseacetatfilter 0,20 µm, Sartorius AG Göttingen). The solutions were filled in to ampoules (10 ml Fiolax klar; OSON Glaswarenfabrik Volkach) and sealed (Füll- und Verschließmaschine Typ R 915, Rota Apparate und Maschinenbau Dr. Henning GmbH & Co). If so designated the batch was sterilised for 15 min at 121 °C at 0.2 MPa (Vakulab S 3000 steam steriliser, Münchner Medizin Mechanik GmbH). The ampoules were stored at RT and in darkness.

4.2. Calibration and extraction of sulphur

4.2.1. Sulphur solutions

A stock solution of sulphur S_8 (Schwefel, puriss., Fluka) dissolved in cyclohexane (cyclohexane, HPLC quality, Baker) was made with a concentration of 5.000 mg/ml. Different volumes were diluted with cyclohexane to obtain concentrations between 100 µg/ml and 1500 µg/ml (Varipette 4810 and Standardtips, Eppendorf-Netheler-Hinz GmbH, Hamburg). The solutions were examined by HPLC as described below.

4.2.2. Thiosulphate solutions spiked with sulphur

100.00 g Sodium thiosulphate pentahydrate was dissolved in 1.00 l purified water to obtain a 10% solution. Different amounts of sulphur (200–1800 µg) were added to 20.0 ml of the thiosulphate solution (balance: Sartorius-Basic^{plus} Elektronische Halbmikrowaage) in the extraction flask (100 ml Erlenmeyer flask with ground stopper). 2.00 ml cyclohexane and 20.0 ml purified water were added. The mixture was shaken on a waterbath for 2 h at a frequency of about 100 min^{-1} at a temperature of 25 °C (GFL-Schüttelwasserbad 1083, Gesellschaft für Labortechnik mbH, Burgwedel). After extraction the flask was allowed to stand in order to separate the two phases. About 20 ml of the aqueous phase was removed carefully with a syringe. The remaining contents of the flask were transferred to a 25.0 ml test tube. After a second standing period a small amount of the organic phase was injected into the HPLC system.

4.2.3. Thiosulphate injection solutions

The contents of the ampoules were poured into the extraction flask (50 ml Erlenmeyer flask with ground stopper) and rinsed twice with 5.00 ml purified water (in-house high purity water system, >16 MΩ). The rinse liquid and 1.00 ml cyclohexane were added. The mixture was shaken on a waterbath for 2 h at a frequency of about 100 min^{-1} at a temperature of 25 °C. The contents of the flask were transferred to a 25.0 ml test tube. After a standing period a small amount of the organic phase was injected into the HPLC system. The commercial preparations were obtained from the pharmacy (10% Natriumthiosulfat-Injektionslösung, 25 × 10 ml, Ch.: 961911, Dr. Franz Köhler Chemie GmbH Alsbach-Hähnlein, Germany; Natriumtiosulfas for inj. 150 mg/ml, 10 × 10 ml, Ch.: 704X2372, Apoteksbolaget Umeå, Sweden; Sodium Thiosulphate 25%, 10 × 20 ml, Ch.: 1201; Pharm. Institut für Forschung und Technologie Pallini Attika, Greece).

4.3. HPLC

The separation was performed with a methanol/cyclohexane (70/30) solvent mixture. 300.0 ml cyclohexane were thus added to 700.0 ml methanol and degassed for 15 min (Bandelin Sonorex RK 100). The column (LiChroCART® 125-4 HPLC Cartridge LiChrosphere 100 RP-18 (5 µm); E. Merck Darmstadt) was equilibrated before first use or for 2 h. Column regeneration was performed by rinsing for 20 min each with cyclohexane, methanol and solvent after one week. The flow rate was 1.0 ml/min, the temperature was ambient. A HPLC system combined with a diode array UV/vis-detector was used (Intelligent Pump L-6200A; Interface L-6000; Diode Array Detektor L-4500; MERCK HITACHI, E. Merck Darmstadt/Hitachi, Ltd. Tokyo; Software: HPLC System Manager Chromatography Data Station Software® Modell D-7000; MERCK HITACHI, E. Merck Darmstadt/Hitachi, Instruments, Inc. San Jose; injection ventil: Rheodyne® 7010, 20 µl sample loop). The solutions were injected to the HPLC system with a 100 µl syringe (Hamilton Gastight® #1710, 100 µl; Hamilton Co., Reno, USA). The detector wavelength was 245 nm (data rate 400 ms).

References

1 Mutschler, E.; Schäfer-Korting. M.: Arzneimittelwirkungen, 7. Ed., p. 822, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart 1996
2 Hündorf, H.P.; Ripp, P.(Eds.): Lehrbuch für präklinische Notfallmedizin Vol. 2, p. 272, Verlagsgesellschaft Stumpf & Kossendey, Edewecht, Wien 1997
3 Marquard, H.; Schäfer, S.G. (Eds.): Lehrbuch der Toxikologie, BI-Wissenschaftsverlag, Mannheim, Leipzig, Wien, Zürich 1994
4 Sefrin, P.: Notfalltherapie, 6. Ed., Urban & Schwarzenberg, München 1998
5 Ludewig, R.; Lohs, K.: Vergiftungen, 8. Ed., Gustav Fischer Verlag, Jena 1991
6 Weger, N. in: Rebentisch, E. (Eds.): Wehrmedizin, p. 266, Urban & Schwarzenberg, München 1980
7 Bukowsi, R.: Eur. J. Cancer **32**, S2 (1996)
8 Europäisches Arzneibuch 1997, 3. Ed., p. 1377. Deutscher Apotheker Verlag, Stuttgart, Govi-Verlag Pharmazeutischer Verlag GmbH, Eschborn 1997
9 The United States Pharmacopeia XXIII, 23th Rev., United States Pharmacopeial Convention, Inc., Rockville 1995
10 Reynolds, J. E. F. (Eds.): Martindale, 31. Ed., Royal Pharmaceutical Society, The Pharmaceutical Press, London 1996
11 Pharmacopoea Helvetica, Deutsche Ausgabe, 7. Ed., Eidgenössische Drucksachen- und Materialzentrale, Bern 1996
12 Bosse, I.: Dissertation, Bayrische Julius-Maximilians-Univerität Würzburg 1991

13 Abschlussbericht E/B41G/M0264/M5912, Bundeswehrkrankenhaus Osnabrück,
14 Chiang, C. H.; Yeh, M. K.; Hsieh, C. H.: Zhonghua yaoxue zazhi (Taipei) **42**, 257 (1990)
15 survey in: Gmelin Institut in Frankfurt am Main (Eds.) in: Gmelins Handbuch der anorganischen Chemie, System Nr. 9 Schwefel Teil B, Lieferung 2, 8. Ed., Verlag Chemie GmbH, Weinheim/Bergstraße 1960
16 Remy, H.: Lehrbuch der Anorganischen Chemie, Vol. I, 13. Ed., Akademische Verlagsgesellschaft Geest & Portig K.-G., Leipzig 1970
17 Bassett, H.; Durrant, R. G.: J.: Chem. Soc., 1401 (1927)
18 Lunge, G.: Chem. Ind. **6**, 298 (1883)
19 Salzer, Th.: Ber. **19**, 1696 (1886)
20 Foerster, F.; Center, K.: Z. Anorg. Chem. **157**, 45 (1926)
21 Jander, G.; Blasius, E.: Lehrbuch der analytischen und präparativen Chemie, 14. Ed., S. Hirzel Verlag, Stuttgart 1995
22 Cherbuliez, E.; Herzenstein, A.: Helv. Chim. Acta **19**, 801 (1936)
23 Hollemann, A. F.; Wiberg, E.: Lehrbuch der Anorganischen Chemie, 91.–100. Ed., Walter de Gruyter, Berlin, New York 1985
24 Raschig, F.: Z. Chem. **33**, 260 (1920)
25 Raschig, F. in: Schwefel- und Stickstoffstudien, Schwefel- und Stickstoffstudien-Verlag GmbH, Leipzig, Berlin 1924
26 Weitz, E.; Spohn, K.: Chem. Ber. **89** 2332 (1956)
27 Budavari, S. (Eds); O'Neil; Smith, A.; Hechelmann, P. E.: The Merck Index, 11. Ed., Merck & Co, Inc., Rahway, N.Y. 1989
28 Steudel, R.; Göbel, T.; Holdt, G.:, Z. Naturforsch. **43b**, 203 (1988)
29 Steudel, R.; Göbel, T.; Holdt, G.:, Z. Naturforsch. **44b**, 526 (1989)
30 Miethe, G.; Surmann, J. P.: Diss. in prep.

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