ASPECTS OF THE ANALYSIS OF

SULPHUR COMPOUNDS IN BEER

by

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SUMMARY

The possible role of volatile sulphur compounds in beer flavour has been investigated using simple analytical methods. These methods were studied and developed particularly for routine quality control use.

Application of the procedures to the analysis of beer volatiles has indicated that, contrary to expectation, sulphur compounds play little part in the flavour/aroma of beers designated as "sulphury".

However the results obtained from this study suggests that a more important aspect of beer sulphur compounds may be in the role of flavour stability. Additionally the presence of dimethyl sulphide in ale has shown it to be at a lower concentration than lager. Since the latter is produced by a different yeast and fermentation the presence of dimethyl sulphide may highlight fundamental differences in the raw materials and process.

Finally suggestions are made for further work on beer flavour stability since this aspect could have fundamental repercussions on production economics and brewing procedures.

INTRODUCTION

Although the influence and importance of flavour in the assessment of food quality has been established and investigated for many years, it is only within the past twenty years that the chemist has been able to examine and determine the nature of some of the compounds which contribute to taste and aroma. The increasing interest in flavour research, particularly in the attempts to correlate aroma with chemical analyses, has been lent impetus from different sources. Examples are, the commercial necessity for maintaining the quality of processed foods and the humanitarian need to provide supplies of sufficient acceptable nutritional food to those areas of the world suffering from malnutrition and famine and lastly but not least the developments which are being made in the application of analytical techniques such as gas chromatography, mass spectrometry, infra red spectrometry, and nuclear magnetic resonance. These techniques permit the separation, identification and measurement of the minute amounts of the flavour compounds present in foodstuffs.

In the brewing industry there has been a similar increasing awareness of the importance of the volatile constituents of beer in relationship to both acceptable and unacceptable flavours. The production brewer is particularly interested in obtaining a greater knowledge of these compounds which affect the quality, and acceptability of his product, since this may lead ultimately to methods for controlling both the process and product quality.

If the conditions under which off-flavours arise during the storage of products can be defined, then the brewer can more certainly specify storage conditions at the sales outlet.

Lawrence[1] in a recent review lists a large number of compounds which contribute to a greater or lesser extent in beer flavour, and Harrison [2] (Table 1) has provided data on the limits of organ-leptic perception for some of the compounds which occur in beer.

TABLE 1 - FLAVOUR THESHOLDS OF BEER COMPONENTS

Compound	Flavour threshold mg l^{-1}	Found in beer mg l^{-1}
n-Propanol	50	15
iso-Butanol	100	15
iso-Pentanol	50	60
Ethyl acetate	25	15
Ethyl isobutyrate	0.1	-
iso-Amyl acetate	2	2
Acetone	> 100	1
2,3-Butanedione	0.1	0.05
2,3-Pentanedione	1.0	0.01
Acetaldehyde	25	10
iso-Butyraldehyde	1	-
iso-Valeraldehyde	0.5	-
Octaldehyde	0.001	-
Hydrogen sulphide	0.005	< 0.001
Ethane thiol	0.01	< 0.01
Dimethyl sulphide	0.03	0.005 - 0.03*

* Values found at Allied Breweries Research Department .

From these figures it is obvious that there are two groups of compounds which, because of their low odour thresholds could have a great influence on aroma. They are volatile sulphur, and carbonyl compounds. The contribution of these compounds to the odour of food has been well-established and investigated since both are responsible for many of the off-flavours which prevail in cooked food-stuffs. It may be assumed that they exert a similar effect on beer flavour should they occur in beer either as fermentation by-products or as a result of degradation reactions which occur during storage.

Many applications of gas chromatography to the analysis of volatile components in beer have been described in the literature (3 - 27) However, the use of sensitive detectors, such as flame ionisation, to demonstrate the presence of trace components, such as carbonyl and sulphur compounds, in beer is impracticable unless other compounds present at much higher concentrations are first removed. Efforts to obtain a complete gas chromatogram of all beer volatiles are, in the author's opinion, of doubtful value. An approach, using more selective methods of analyses, provides information more relevant to flavour studies.

In following the idea of specific or selective analyses for flavour studies, the present work has been particularly concerned with the estimation of volatile sulphur compounds in beer. This was because it was known that the volatile sulphur compounds are present in only minute amounts and the application of both specific

and sensitive methods would be essential. Two approaches to the sulphur problem are possible.

1. A combination of the resolution possible by gas chromatography with detectors possessing both sensitivity and a specific response to sulphur. Two such detectors are available which could be used in this context, namely the flame photometric detector described by Brody and Chaney[28] and a microcoulometric detector proposed by Coulson[29]. These detectors would be of particular value in the determination of hydrogen sulphide and alkane thiols in beer vapour. The application of the flame photometric detector to the determination of beer sulphur volatiles has recently been described by Barwald[30].
2. The alternative approach is to use methods which depend on selective isolation and application of specific and sensitive colour reactions. The advantage of such procedures is that no sophisticated or expensive equipment is necessary and the analyses can be readily performed in routine analytical laboratories. This approach was chosen for the present study.

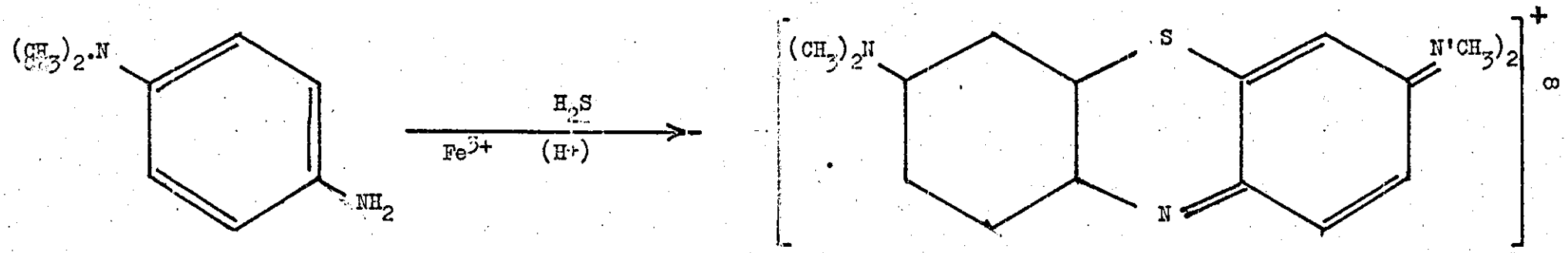
CHAPTER 1 - THE DETERMINATION OF HYDROGEN SULPHIDE

1.00 INTRODUCTION

The occurrence of hydrogen sulphide in beer has been known for a considerable time but the first systematic investigations of hydrogen sulphide production during fermentation were made comparatively recently by Ricketts and Coutts[31 - 33]. Subsequently, the formation and interaction of hydrogen sulphide during fermentation has been investigated by other workers.

Methods for the determination of hydrogen sulphide are described in the literature [34 - 39], but relatively few of these are satisfactory for estimating the low concentration of hydrogen sulphide which exists in beer. However, a rapid and selective method based on micro-coulometry[29] and a gas chromatographic method using a flame photometric detector[28] have recently been described. Of the many colorimetric methods available, that based on the formation of methylene blue has been claimed to be the most sensitive and specific for the assay of low concentrations of hydrogen sulphide. This reaction, which proceeds as shown in Fig. 1.1, was first described by Emil Fischer[40] and applied by Brenner *et al.*[41 - 43] to beer analyses. This latter work has been criticised by Jansen[44] who found that "It did not give satisfactory results" and concluded that it could only be applied to beers of low copper content (less than 0.05 mg l^{-1}).

FIG. 1.1 - FORMATION OF METHYLENE BLUE



Although the methylene blue method for determining hydrogen sulphide has been used empirically for many years, the method had not been investigated thoroughly until the recent work of Gustafsson [45] who described attempts to attain optimum reaction conditions.

Comparison of the results obtained early in the present study with those of Gustafsson showed excellent agreement in the effect on colour development of pH, temperature, concentration of reagents and their mode of addition. The reaction conditions specified by Gustafsson have, therefore, been adopted and applied to the study of the determination of hydrogen sulphide in beer and the effects of storage time, temperature and trace metal content evaluated.

1.20 EXPERIMENTAL

1.21 Reagents

All reagents were of AR or NAR grade unless otherwise specified. All solutions were prepared with specially purified water.

Para-amino-dimethylaniline sulphate solution (0.005 M): p-amino-dimethylaniline sulphate (0.93 g) was dissolved in sulphuric acid (3.5 M) and diluted to one litre with sulphuric acid (3.5 M).

Zinc acetate reagent solution: zinc acetate (0.25 M) in sodium acetate solution (0.1 M). Traces of heavy metals were removed by precipitation as sulphides. A freshly prepared sodium sulphide solution (2 ml of 0.05 M), was added dropwise with shaking to zinc acetate solution (1 litre). After standing overnight the precipitate was removed by filtration and the filtrate stored in a glass stoppered bottle.

Iron (III) ammonium sulphate solution: iron (III) ammonium sulphate (0.25 M) in sulphuric acid (0.5 M).

Constant boiling hydrochloric acid solution (21% w/w): concentrated hydrochloric acid (36% w/w) was distilled at atmospheric pressure through a borosilicate glass still. The constant boiling fraction was collected and used in all analyses.

Nitrogen: 'oxygen-free' grade was further purified by passing through a solution of alkaline pyrogallol and then through potassium permanganate solution (2.5% w/v) saturated with mercury (II) chloride.

L-ascorbic acid (solid): complied with the British Pharmacopoeia (1968).

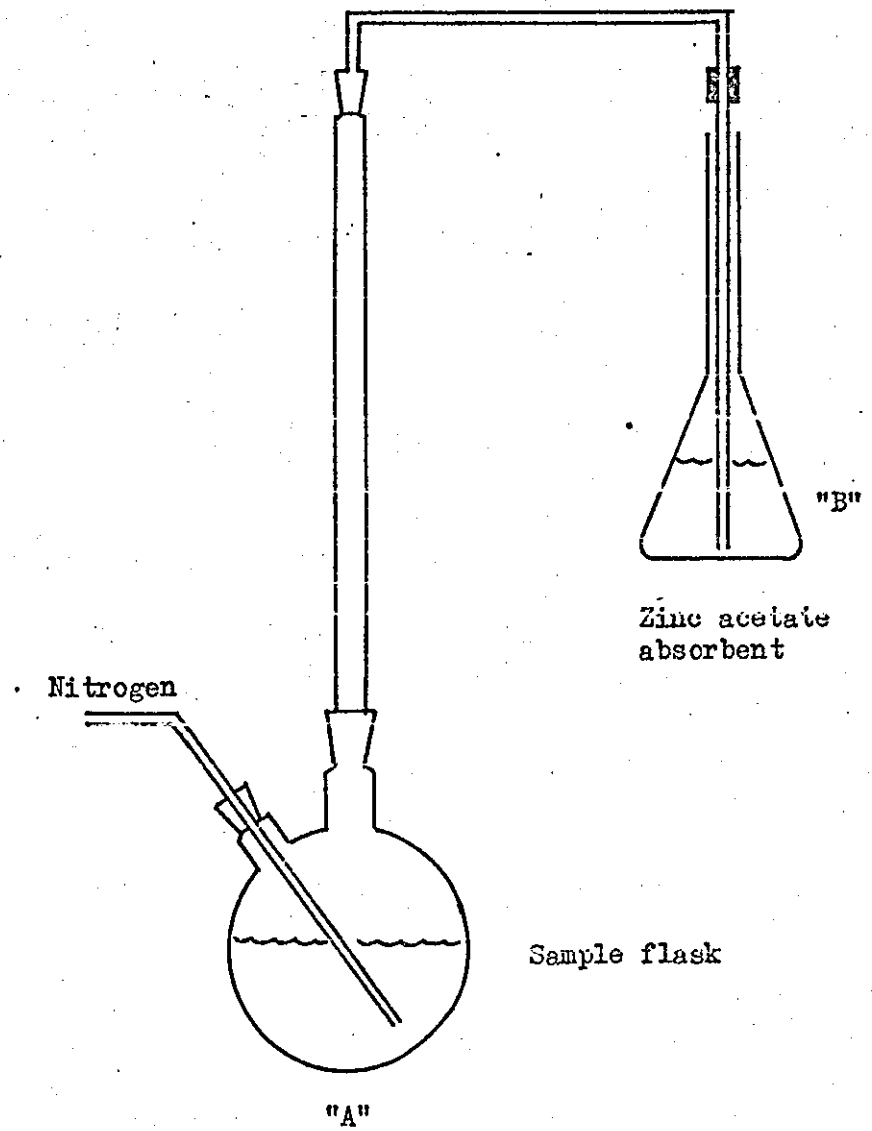
Methylene blue: special laboratory reagent grade (assay 96%) supplied by Fisons Scientific Ltd.

Purified water for preparation of reagents: distilled and deionized water (3 l) obtained from an ion-exchanger apparatus (Elgastat) was purified by distillation from barium chloride (30 g) and potassium permanganate (10 g). The distillation flask was fitted with a fractionation column (length 550 mm x diameter 38 mm), packed with Raschig rings (6 mm). The apparatus was allowed to 'steam' for ten minutes before collecting the distillate, the first 200 ml of which was discarded.

1.22 Apparatus

1. The gas purging unit is shown in Fig. 1.2.
2. "Uvispek" Spectrophotometer and 10 mm glass cells.

FIG. 1.2 - APPARATUS FOR COLLECTION OF HYDROGEN SULPHIDE



1.30 PROCEDURE

1.31 Collection of Hydrogen Sulphide

Free hydrogen sulphide: A sample of beer (500 ml) was placed in the flask (A) of the purging apparatus (Fig. 1) which had been previously flushed with nitrogen. Ascorbic acid (0.5 g) was added to the sample which was then purged with nitrogen for one hour at 25°C, at a flow rate of 150 ml per minute. The hydrogen sulphide in the gas stream was absorbed in trap B, a volumetric flask (100 ml) containing zinc acetate reagent solution (10 ml) and deionized water (70 ml). The contents of the trap were assayed using the colour development procedure described below.

Bound hydrogen sulphide: The procedure was the same as described above with the addition of hydrochloric acid (220 ml of 21% w/w) to the sample before the start of gas purging. Colour development of the contents of trap B is described below.

1.40 COLOUR DEVELOPMENT

After absorption of the hydrogen sulphide, trap B was placed in a water bath, 20°C ± 1°C, for ten minutes. Para-amino-dimethylaniline solution (10 ml) was added so that it formed a layer in the bottom of the flask. The contents were then gently mixed by swirling. Any zinc sulphide adhering to the delivery tube was dissolved by sucking some of the solution

up into the tube. Iron (III) ammonium sulphate solution (2 ml) was added, the flask stoppered and the contents vigorously shaken for thirty seconds. The solution was finally diluted to 100 ml with distilled water and after standing at 20°C for fifteen minutes, the absorbance was measured at 667 nm (the wavelength of maximum absorbance of the methylene blue formed in the reaction) against distilled water.

A reagent blank was determined by purging a sample of the purified water with nitrogen and passing the gas through zinc acetate reagent. The colour development was as described and the absorbance subtracted from the absorbance of the sample. The hydrogen sulphide content was obtained from either a calibration graph or by using a calibration factor, F, based on the measured extinction coefficient.

$$H_2S \text{ content } (\mu g/l) = \frac{\text{Absorbance at 667 nm} \times 10^6 \times 1000}{F \times \text{vol. of sample}}$$

$$\text{where } F = \frac{\text{Absorbance at 667 nm} \times 10^6}{H_2S \ (\mu g)}$$

The value for F was found to be 11,500 (± 4.7%) based on the conversion of known weights of hydrogen sulphide to methylene blue.

1.50 STANDARDISATION

Two calibration procedures were used. The first, based on hydrogen sulphide solutions, was used routinely. An alternative procedure using sodium sulphide was occasionally used for cross checking purposes.

1.51 Hydrogen sulphide

Hydrogen sulphide was passed through distilled water (500 ml) for five seconds. An aliquot (5 ml) of this solution was added to zinc acetate reagent (20 ml), care being taken to keep the pipette below the surface of the reagent. The colloidal suspension of zinc sulphide was diluted to 100 ml with zinc acetate reagent, portions (1 - 10 ml) were then added to volumetric flasks (100 ml) and the volume made up to 10 ml by adding the appropriate volume of zinc acetate solution. The methylene blue colour was then developed using the procedure described above. The remaining hydrogen sulphide water (495 ml) was assayed by iodimetric titration.

1.52 Sodium sulphide

Sodium sulphide stock solution (0.5 M) was used to prepare standard sulphide solutions. It was found necessary to standardize the stock solution immediately before the preparation of a calibration curve because of the instability of aqueous sodium sulphide. This solution was standardized as follows:- Sodium sulphide stock solution (10 ml) was added to the standard iodine solution (20 ml of 0.100 M). Deionized water (100 ml) and hydrochloric acid (1 ml of 3 M) were added and the unreacted iodine titrated with standard sodium thiosulphate (0.100 M). The hydrogen sulphide content was calculated as before.

1.60 RESULTS

1.61 Linearity, reproducibility and stoicheiometry

Standard hydrogen sulphide solutions were prepared and assayed over the range 0 - 80 micrograms of hydrogen sulphide. Agreement with the Beer-Lambert law was shown by a linear plot over the range 0 - 40 micrograms of hydrogen sulphide. The results are given in Table 1.1 and shown graphically in Fig. 1.3.

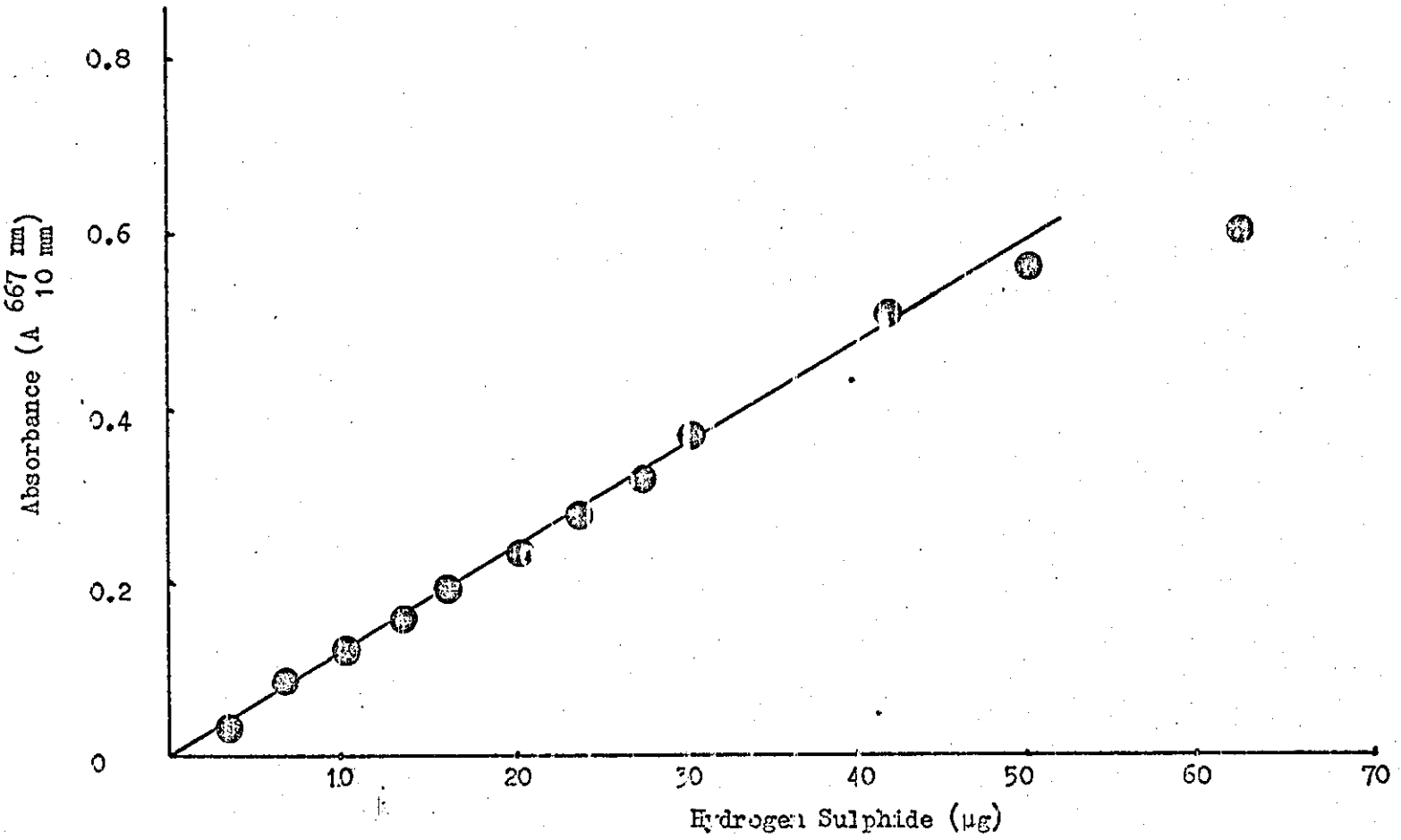
The non-linearity of the response above 40 μg H_2S is due to dimerisation of methylene blue in acid solution[45].

The calibration factor (F) for the direct conversion of hydrogen sulphide to methylene blue was evaluated. The individual values of F are also shown in Table 1.1. The mean value of F for the linear portion was 11.5×10^{-3}. The standard deviation observed corresponds to a coefficient of variation of 4.7%.

TABLE 1.1 - CONFIRMATION OF BEER-LAMBERT LAW AND CALIBRATION FACTOR

Hydrogen sulphide (micrograms)	Absorbance (1 cm 667 nm)	'F'
3.4	0.036	10.54×10^{-3}
6.8	0.083	12.15
10.3	0.117	11.42
13.7	0.158	11.56
17.1	0.195	11.42
20.5	0.234	11.42
23.9	0.275	11.50
27.3	0.313	11.45
30.7	0.375	12.20
34.2	0.388	11.36
Mean value for F		11.50
Standard deviation		0.457
Coefficient of variation (%)		4.7

FIG. 1.3 - PLOT SHOWING AGREEMENT OF CONVERSION OF HYDROGEN SULPHIDE TO METHYLENE BLUE WITH THE BEER-LAMBERT LAW



Using this calibration factor, a mean coefficient of variation of 4% was found for replicate analyses (9 results) of hydrogen sulphide at two concentrations (6 and 45 µg per litre). The results are given in Table 1.2. It should be noted that these results are more variable than those obtained by Gustafsson for a similar series. This is attributed to the influence of variation in laboratory temperature on the absorbance measurements.

TABLE 1.2 - REPRODUCIBILITY OF METHOD AT TWO CONCENTRATIONS

	Standard 1 (6 µg H_2S per litre)	Standard 2 (45 µg H_2S per litre)
	6.95	46.8
	6.35	46.8
	5.92	45.2
	6.16	45.7
	5.92	45.2
	6.70	47.3
	6.35	46.0
	6.35	46.0
	6.35	42.2
Mean	6.34	45.6
Standard deviation	0.333	1.496
Coefficient of variation (%)	5.2	3.3

The yield of methylene blue from hydrogen sulphide was determined by comparing the $E^{1\%}_{1cm}$ values for both authentic methylene blue ($E^{1\%}_{1cm}$ = 1794) and that prepared from known amounts of hydrogen sulphide (Table 1.3) under the conditions of analysis.

The measured $E^{1\%}_{1cm}$ value for methylene blue derived from hydrogen sulphide was 1375, the percentage conversion of hydrogen sulphide to methylene blue is thus $\frac{1375}{1794} \times 100 = 76.5\%$. Because the methylene blue was 96% pure, a yield of 73% was obtained, which is in reasonable agreement with the value of 68% obtained by Gustafsson.

TABLE 1.3 - ABSORBANCE OF METHYLENE BLUE SOLUTIONS

Methylene blue (micrograms)	Absorbance (10 mm, 667 nm)	$E^{1\%}_{1cm}$
73	0.137	1870
148	0.275	1860
222	0.398	1795
296	0.517	1750
370	0.626	1790
444	0.797	1795
Average value for $E^{1\%}_{1cm}$ = 1794		

1.62 Isolation Procedure

In the method described by Brenner[41-43], hydrogen sulphide is expelled from beer which was acidified to a pH less than 1. When this procedure was re-examined, problems due particularly to acidification became evident. Preliminary experiments to determine the effect of temperature and pH on the recovery of hydrogen sulphide from aqueous ethanol solutions were performed, using the following model systems to which a known amount of hydrogen sulphide (40 µg) had been added.

1. Concentrated hydrochloric acid (200 ml of 21% w/w) was diluted to 500 ml with aqueous ethanol (5% v/v) at 25°C or 50°C.
2. Aqueous ethanol (500 ml of 5% v/v) at 25°C or 50°C.

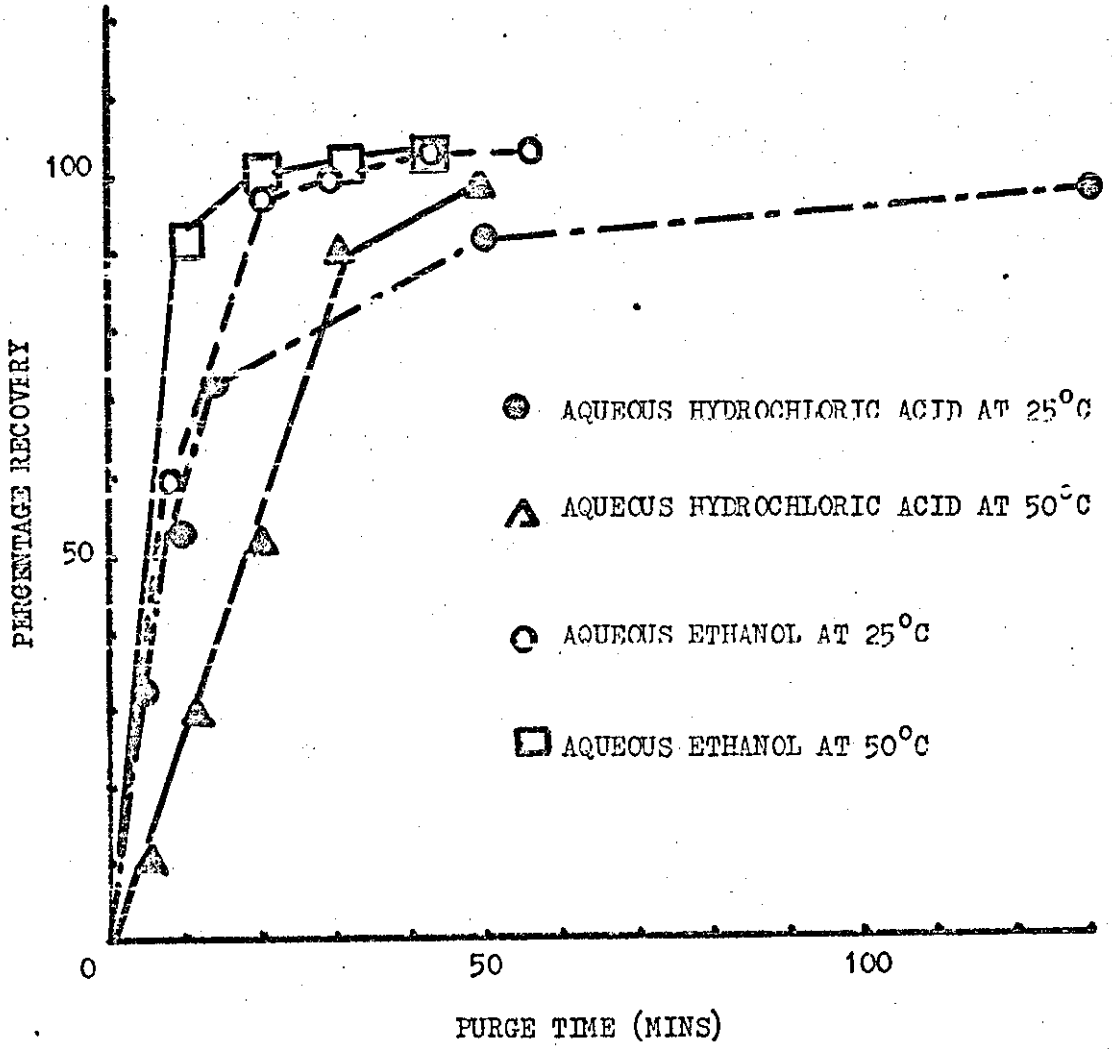
From the results, shown diagrammatically in Fig. 1.3 the best system for recovering hydrogen sulphide is system 2, the non-acidified ethanolic solution.

By using this model system, the recovery of hydrogen sulphide was shown to be satisfactory at various H_2S concentration levels (Table 1.4), whereas the addition of hydrochloric acid required an extension of the purge time to two hours to obtain the same recovery. Because of the shorter analysis time obtained, purging at 25°C for one hour without addition of hydrochloric acid was adopted in the future assays.

TABLE 1.4 - RECOVERY OF HYDROGEN SULPHIDE FROM AQUEOUS ETHANOL (5% V/V AT 25°C)

Hydrogen sulphide added (µg)	Hydrogen sulphide found (µg)	Recovery (%)
7.5	7.3	97.6
10.8	10.8	100.0
14.6	14.6	100.0
16.7	15.8	94.8
22.4	22.4	100.0
27.2	26.8	98.4
Average Recovery = 98.9%		

FIG. 1.3 - RECOVERY OF HYDROGEN SULPHIDE FROM MODEL SYSTEMS



1.63 Examination of possible interferences

The extent of interference due to volatile sulphur compounds, other than hydrogen sulphide, occurring in the beer as fermentation by-products, was assessed by determining both the effect on the formation of methylene blue and the extent to which the compounds were expelled along with hydrogen sulphide from a model aqueous ethanol system.

The effect of sulphur compounds on methylene blue colour development was established by adding known amounts of sulphur dioxide, ethane thiol, dimethyl sulphide and diethyl disulphide to volumetric flasks (100 ml) each of which contained a known amount of hydrogen sulphide and zinc acetate reagent solution.

A second series was prepared containing only the hydrogen sulphide standards and zinc acetate reagent solution. The hydrogen sulphide in the two sets of samples was then determined using the recommended colour development procedure. In flasks containing the sulphur compounds, other than hydrogen sulphide, the development time was extended from fifteen to forty five minutes to ensure maximum absorbance and interference.

The results, given in Table 1.5, confirm that an apparent loss of hydrogen sulphide occurs when sulphur volatiles are present during colour development. That the reduction in the methylene blue colour is due to the presence of sulphur dioxide was confirmed by the results in Table 1.6, these results also demonstrate that sulphur dioxide must be present at a minimum

concentration at the colour development stage before interference occurs.

TABLE 1.5 - EFFECT OF VOLATILE SULPHUR COMPOUNDS ON FORMATION OF METHYLENE BLUE

(a) Mixture of sulphur dioxide (8 mg), ethane thiol (25 µg), dimethyl sulphide (25 µg), diethyl disulphide (25 µg).

(b) Mixture of ethane thiol (25 µg), dimethyl sulphide (25 µg), diethyl disulphide (25 µg).

(c) Sulphur dioxide (8 mg).

Mixture of sulphur compounds present	Hydrogen sulphide added (µg)	Hydrogen sulphide found (µg)	Recovery %
(a)	6.8	2.8	41.2
"	14.5	5.1	35.2
"	20.9	8.5	40.6
"	27.9	10.1	36.2
"	32.4	11.7	36.2
(b)	23.3	23.4	100.0
(c)	23.3	10.9	46.8

TABLE 1.6 - EFFECT OF SULPHUR DIOXIDE ON METHYLENE BLUE FORMATION

(17.6 µg H_2S added to all determinations)

Sulphur dioxide added (µg)	Hydrogen sulphide found (µg)	Recovery %
0	17.6	100
0.2	17.6	100
0.4	17.4	99
0.8	17.4	99
1.6	17.2	98
2.5	16.5	94
4.1	16.3	92.5
4.9	15.2	86.5
6.6	10.8	61.4
8.20	8.7	49.4

The extent to which sulphur dioxide and other sulphur volatiles could be expelled from a beer sample by the nitrogen purging technique, was assessed by adding the volatile sulphur compounds (as for Table 1.5) to an aqueous ethanol solution (5% w/v) of hydrogen sulphide. By this increase in the concentration of the added volatiles above the normal expected limit, the possibility of interference was considerably exaggerated. Determination of the recovered hydrogen sulphide under these conditions showed that there was little or no loss of hydrogen sulphide, demonstrating that the carry over of sulphur dioxide is insufficient to interfere with methylene blue colour formation. The results obtained are given in Table 1.7.

TABLE 1.7 - RECOVERY OF HYDROGEN SULPHIDE IN PRESENCE OF VOLATILE SULPHUR COMPOUNDS

Hydrogen sulphide added (µg)	Hydrogen sulphide found (µg)	Recovery %
6.8	7.1	104
8.3	7.7	93
10.7	10.7	100
11.8	11.1	94
16.4	15.3	94
17.0	15.6	92
17.7	17.0	96
20.7	20.7	100
29.6	28.5	96
32.7	33.6	103

1.70 HYDROGEN SULPHIDE CONTENT OF BEER

Pasteurized, bottled beers were analysed to establish normal levels for the hydrogen sulphide content (Table 1.8). Pasteurized beer was chosen for the experiment to eliminate the effect of yeast in producing hydrogen sulphide as a fermentation by-product. Similarly, a series of beers which had been stored at 0°C and 20°C, for taste panel evaluation, was analysed. The beer stored at 20°C had been rejected by the taste panel because of flavour breakdown (Table 1.9).

The results obtained indicated that little free hydrogen sulphide occurs in either fresh beer or beer stored at 20°C. However, in those samples stored at 20°C the hydrogen sulphide liberated upon acidification showed significant increases.

TABLE 1.8 - HYDROGEN SULPHIDE CONTENT OF BEER

Sample	Original Gravity °P	Free H_2S (µg/L)	Bound H_2S (µg/L)
Draught Ale	9.25	0.3	46.0
	9.25	0	18.1
	9.25	0.2	24.6
Bottled Ale	12.0	0.2	3.4
	12.0	1.0	11.2
	10.5	0.7	12.0
	10.5	0	0.8
	12.0	0	39.2
Bottled Lager	7.5	0.2	9.7
	11.0	0.2	8.3
	11.0	0	3.6
	11.0	0	8.6
	9.0	0	0.7

The original gravity of beer is defined as the specific gravity of the wort from which that beer has been fermented.

°Plato (°P) - the percentage of sucrose by weight, or volume, corresponding to the specific gravity of a wort or beer, measured at 17.5°/17.5°C.

TABLE 1.9 - HYDROGEN SULPHIDE CONTENT OF STORED BEER

Sample	Original gravity	Storage temperature			
		0°C		20°C	
	°Sacch	Free	Bound	Free	Bound
		(all results in µg/litre)			
Canned ale	48	0.2	1.4	0.3	16.6
Canned ale	48	0	1.5	0.3	13.1
Canned lager	48	0	1.0	1.0	10.8
Bottled lager	48	0	1.7	0	12.7

Because of this latter effect, a more detailed examination of the factors which influence this 'bound' hydrogen sulphide was commenced and, since the bound hydrogen sulphide may be related to flavour stability, all experiments were performed on beer which had been pasteurized and bottled.

1.71 The effect of storage time and temperature

The increase in bound hydrogen sulphide during storage was investigated further by storing bottles of pale ale (OG 1048) and lager (OG 1044) at 0°C and 20°C for periods up to twelve weeks. These were analysed at two-weekly intervals and the change in hydrogen sulphide concentration was followed (Fig. 1.4). Taste panel observations were also obtained in an attempt to correlate flavour changes with bound hydrogen sulphide. In all instances the taste panel rejected the beer stored at the higher temperature; this also had the highest total hydrogen sulphide. It is difficult to obtain decisive correlation between bound hydrogen sulphide and flavour stability unless the production

of other off-flavours can be avoided. Nevertheless, the production of bound hydrogen sulphide during storage may be symptomatic of a more fundamental change in a sulphur-bearing precursor which is implicated in flavour stability.

Confirmatory experiments on the effect of temperature were performed by storing beer at temperatures from 0°C to 60°C and following the hydrogen sulphide content by daily analysis. As found previously, bound hydrogen sulphide concentration increased with increase in storage temperature (Fig. 1.5) but with no change in free hydrogen sulphide content.

1.72 Influence of metal ions

Jansen[44] has previously discussed an attempted application of Brenner's method for hydrogen sulphide assay to beers which contain copper. It was considered to be of interest to examine this and similar systems.

Investigation of the effect of temperature and additional metal ions showed that the observed results were due to induced variation in samples, rather than to failure in the analytical procedure.

Ale was treated with either copper (II) acetate, iron (III) chloride or silver nitrate solution to give cation concentrations of 10 mg/litre. The samples were incubated in the absence of light and oxygen at 20°C and 40°C along with corresponding controls, which contained no added cation, and were analysed daily for free and bound hydrogen sulphide.

FIG. 1.4 - EFFECT OF STORAGE TIME AND TEMPERATURE ON BOUND HYDROGEN SULPHIDE

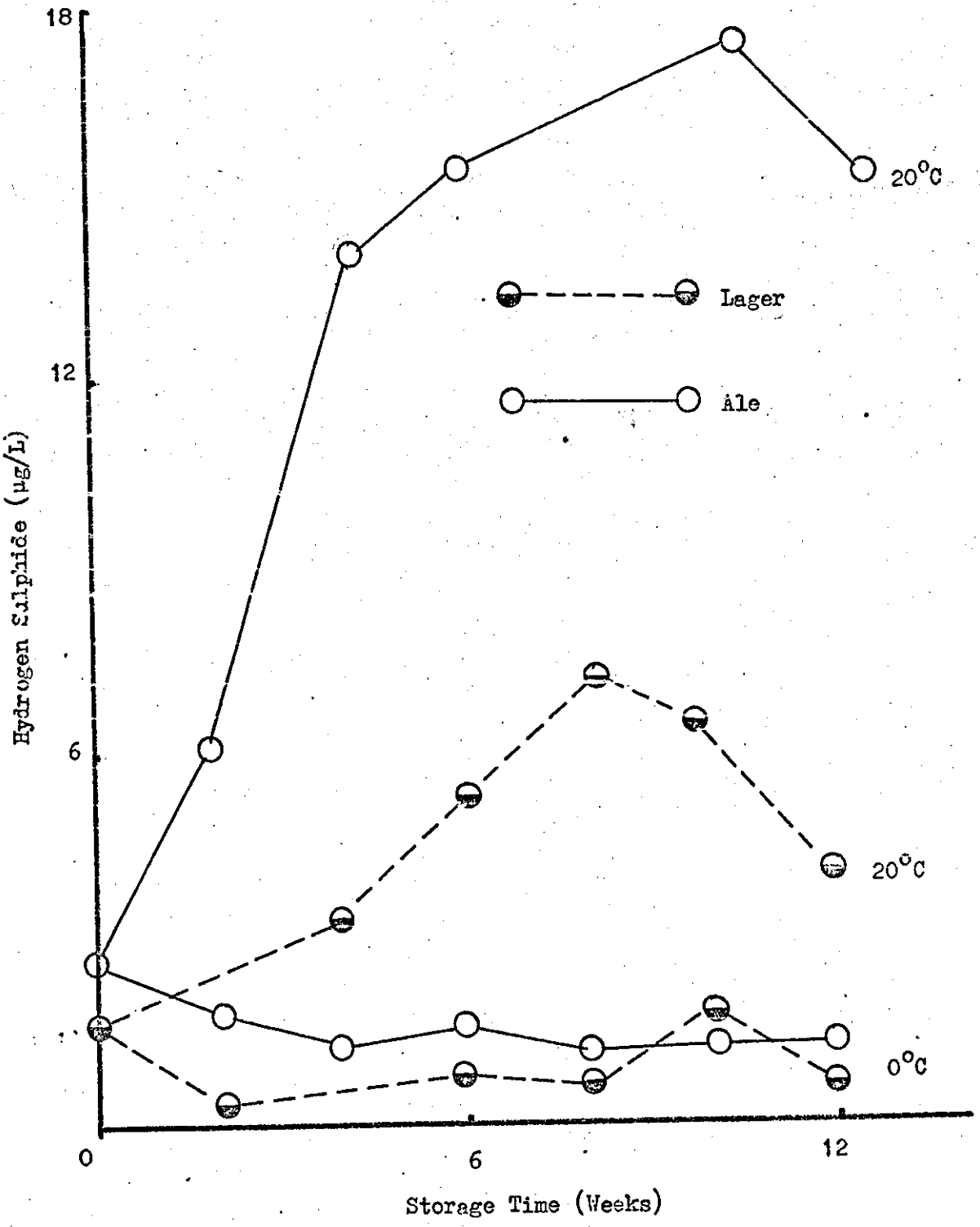
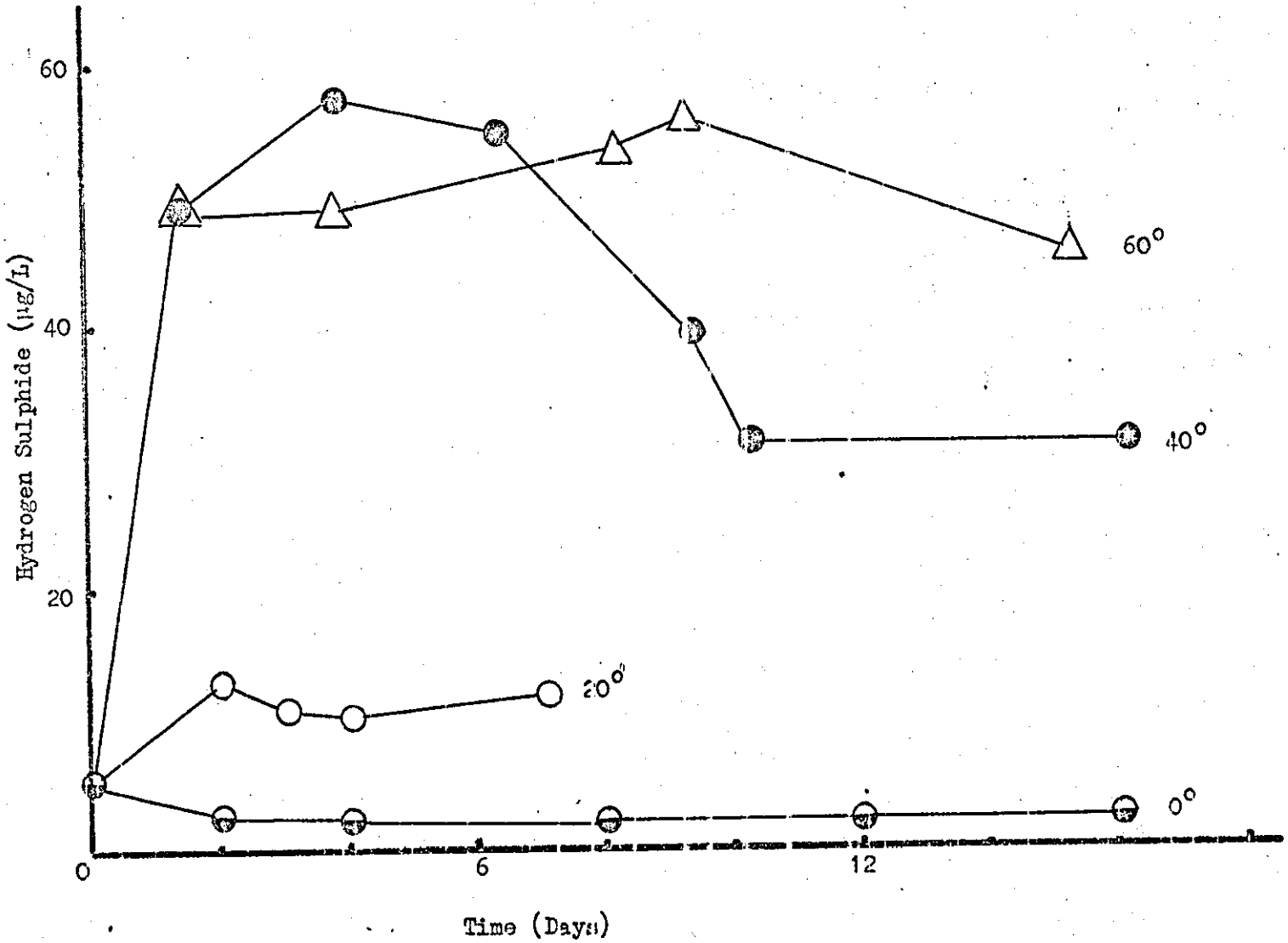


FIG. 1.5 - EFFECT OF TEMPERATURE ON BOUND HYDROGEN SULPHIDE



The results illustrated in Fig. 1.6 show the relative effectiveness of silver, copper (II) and iron (III) in promoting bound hydrogen sulphide formation at 20°C and 40°C. By increasing the metal content to 100 p.p.m. even greater amounts of bound hydrogen sulphide were produced (> 500 µg per litre for Copper (II)).

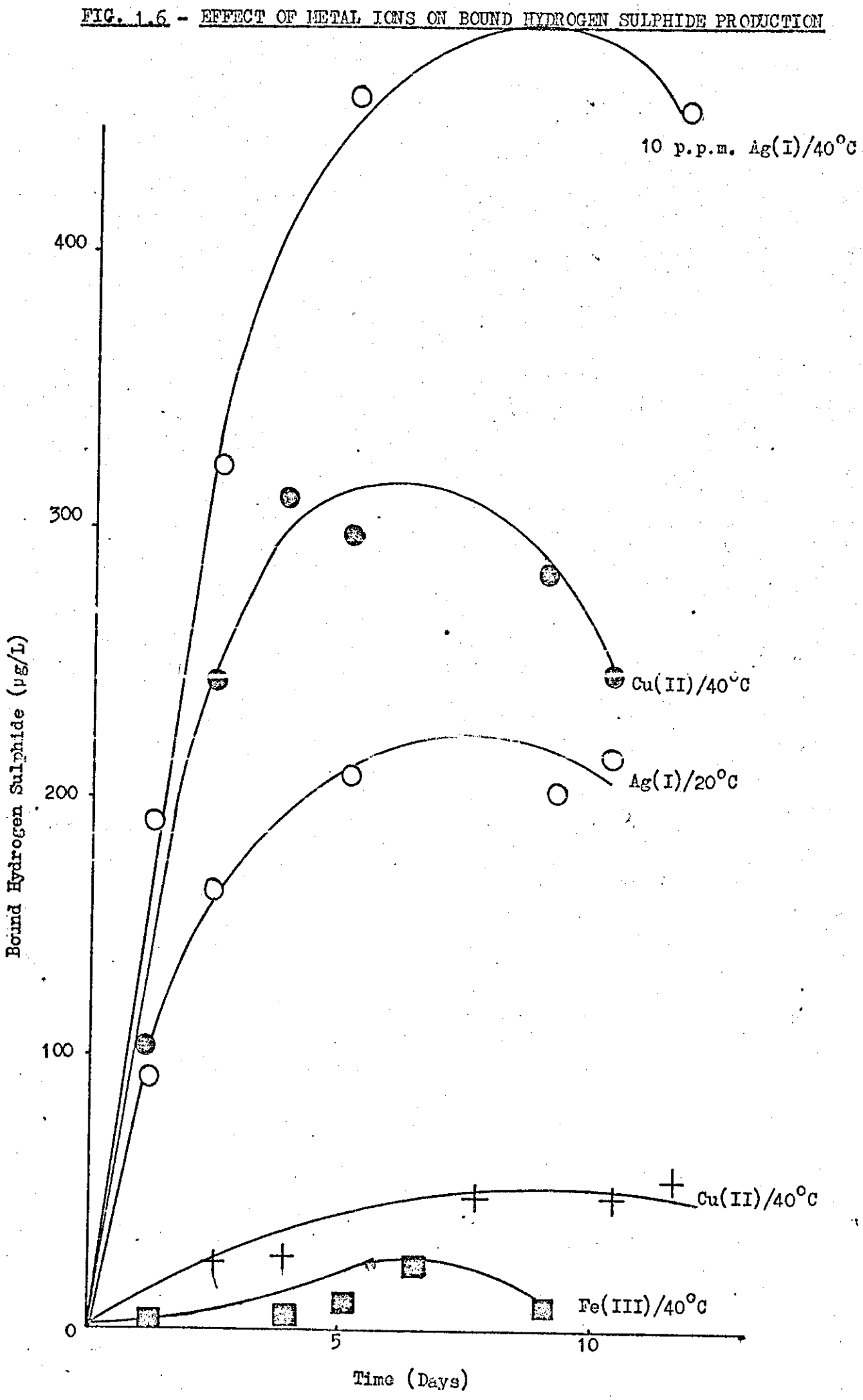
The catalytic effect was eliminated by addition of sodium ethylene diamine tetra-acetate as shown by the results reported in Table 1.9. These data suggest that the metals participate by complexation with a sulphur component present in the beer.

TABLE 1.9 - BOUND HYDROGEN SULPHIDE IN PRESENCE OF EDTA AND COPPER AT 20°C

Time (days)	Control	Control + 10 p.p.m. Cu^{++}	Control + 10 p.p.m. Cu + 10 p.p.m. EDTA	Control + Cu/EDTA Complex
(all results quoted as µg per litre)				
0	0.4	0.4	0.4	0.4
1	0.7	50.8	0	0
2	0	61.9	0	0
3	-	-	-	-
4	0	102.9	0	0

0 = No free hydrogen sulphide detected

FIG. 1.6 - EFFECT OF METAL IONS ON BOUND HYDROGEN SULPHIDE PRODUCTION



1.80 DISCUSSION

Most analytical methods can be divided into two distinct operations, namely, an isolation or concentration stage followed by a quantitative measurement. In the method under investigation the latter measurement was performed by conversion of the hydrogen sulphide to methylene blue. This reaction was examined to establish the effect of pH, temperature and mode of reagent addition on the stoicheiometry of the reaction. During this investigation the results of a study by Gustafsson on the formation of methylene blue were obtained. Because of the agreement between the two findings, the procedure described by Gustafsson was adopted with only minor modifications.

The effect of interference from other volatile sulphur compounds likely to occur in beer, on the formation of methylene blue was investigated and found to be insignificant, except for sulphur dioxide. Nevertheless, the extent to which this latter compound can be expelled from beer by nitrogen purging was found to be so negligible that the possibility of this interference can be ignored if the procedure described is used.

The major problems relevant to the determination of hydrogen sulphide in beer occur in the isolation procedure. In the method described by Brenner the hydrogen sulphide is expelled from beer which has been acidified to a pH less than one. This immediately raises questions of artefact formation

due to acid hydrolysis of labile intermediates. Jansen has further criticized this method as being applicable only to beers of low copper content.

By examining model systems optimum conditions for the removal of hydrogen sulphide from aqueous ethanol solutions have been established. Application of these conditions to beer demonstrated that little free hydrogen sulphide was present, whereas acidification of the sample, as recommended by Brenner, produced large amounts of hydrogen sulphide, indicating that the hydrogen sulphide was bound in some manner within the beer. Whether this bound hydrogen sulphide can be regarded as an index of 'sulphidic' aroma in beer is questionable since it is reasonable to assume that only free hydrogen sulphide will influence aroma.

An investigation of the variables influencing the formation of bound hydrogen sulphide showed that storage time, temperature and the presence of a metal ion, were responsible for the increased concentrations of hydrogen sulphide liberated by acidification. One conclusion arising from these findings is that an acid-labile intermediate is produced on storage of beer, and its formation is catalyzed by trace metals, particularly silver, copper (II) and iron (III). A similar conclusion was reached by Jansen who postulated the formation of a copper complex. The apparent catalytic effect of metal ions eliminates

the possibility of decomposition of a metal sulphide since the addition of metal ions would not increase the available hydrogen sulphide. This is especially true for silver, which has the greatest catalytic effect and whose sulphide is not readily decomposed by hydrochloric acid at normal temperatures.

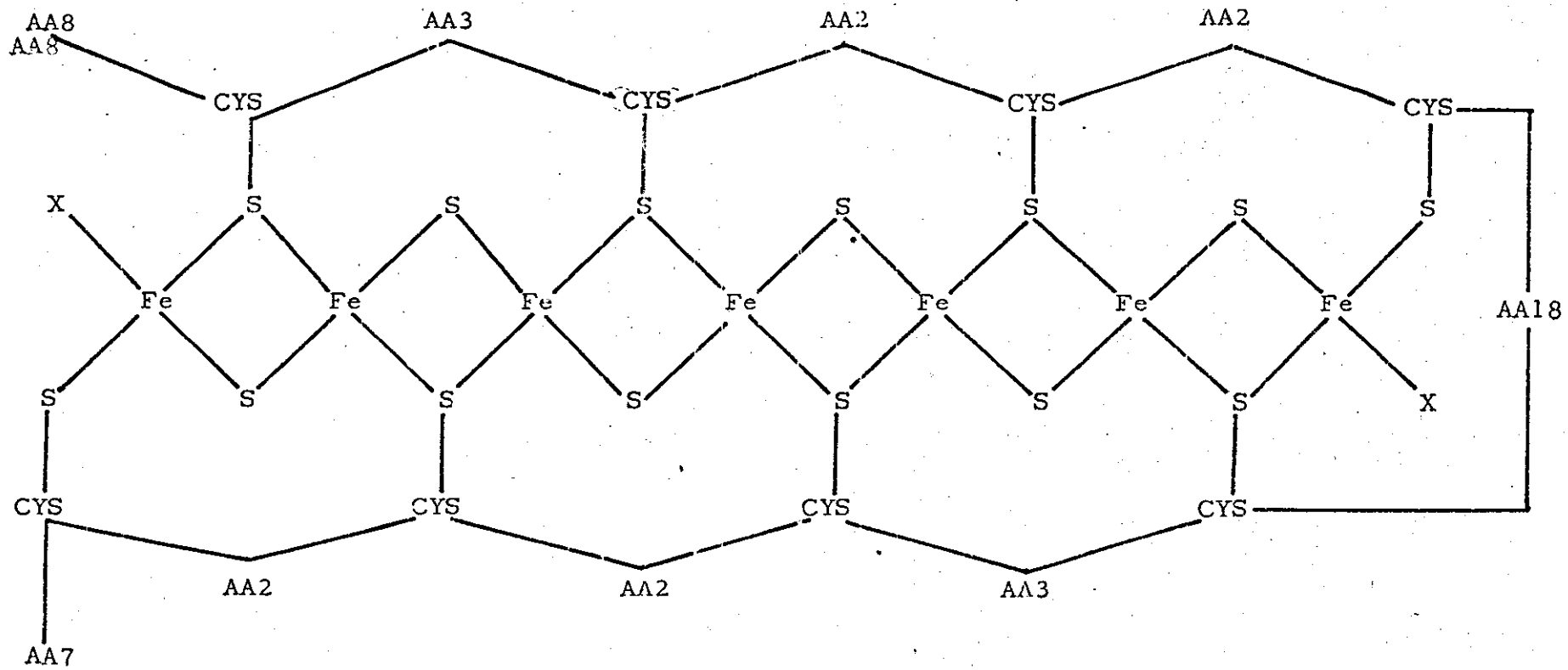
The production of bound hydrogen sulphide in pasteurized bottled beer at a temperature of 20°C and its apparent increase with time is of considerable interest, since it may be related in some way to flavour stability and shelf life. If the reaction mechanism can be defined then it might be possible to develop a method for the accurate prediction of both flavour stability and shelf life. An attempt to explain the reaction mechanism was carried out using a variety of model systems containing cystine, cysteine, methionine and glutathione, in the presence and absence of silver and copper ions. No hydrogen sulphide was liberated in any of these experiments. Nevertheless, in order to account for the production of hydrogen sulphide in beer the sulphur must be present as either divalent $S^{=}$ or monovalent SH^{-} possibly existing as an organometallic complex which produces hydrogen sulphide upon acid hydrolysis.

An example of an organometal complex which releases hydrogen sulphide on acid hydrolysis is the electron-transport protein ferredoxin (Fig. 1.7). Recent investigations[46 - 48] on this compound have shown it to contain seven iron (III) ions

complexed with cysteine residues in the peptide chain. This structure which contains labile iron-sulphur bonds readily forms hydrogen sulphide upon hydrolysis, but nevertheless controversy exists as to the exact mechanism for the release of hydrogen sulphide. β-Elimination from cysteine is favoured by Bayers[48] whereas Malkin and Rabinowitz[49] oppose this hypothesis. While it is not suggested that ferredoxin is responsible for the observed reaction of beer, it is possible that a similar stereochemical relationship exists between the sulphur (or sulphydryl) residues of a beer peptide and a metal ion. Some evidence for this is shown by the results which demonstrate that the effectiveness of a metal in producing bound hydrogen sulphide decreases in the order Ag>Cu>Fe. This series is similar to the order of stability constants for the complexes of these metals with certain sulphur amino acids (cystine, cysteine, methionine)[50].

Because of the possible relationship between the production of hydrogen sulphide by acid hydrolysis, breakdown of sulphur linkages on storage, and flavour stability it will be of great interest to establish the nature of this labile intermediate. Further studies in this difficult field are thus indicated.

FIG. 1.7 - STRUCTURE OF FERREDOXIN (TANAKA et al.)



CHAPTER 2 - THE DETERMINATION OF DIMETHYL SULPHIDE IN BEER

2.00 INTRODUCTION

The importance of dimethyl sulphide to the flavour of various foods has become apparent over the past few years. Because of the low flavour threshold - values of 12 µg per litre in water[51], 19 µg per litre in milk[52-53] and 30 µg per litre in beer[54-55] have been reported - it can contribute to flavour at very low concentrations.

Its presence has been detected in a variety of foodstuffs e.g. tea, tomatoes, milk, asparagus and cabbage [56-. In general the formation of dimethyl sulphide in foodstuffs has been ascribed to the thermal degradation of a precursor, namely S-methyl methionine sulphonium salt, according to the sequence[61].

$$(CH_3)_2.\dot{S}:CH_2.CH(NH_s).CO_2H \longrightarrow (CH_3)_2.S + CH_2.(OH)CH_2.CH(NH_2)CO_2H$$

Although the presence of dimethyl sulphide in beer has been reported by Ahrenst-Larsen & Hansen[54] and Kepner et al.[55], its quantitative determination and influence on aroma has received scant attention. In addition the mechanism for its formation in beer has not been elucidated since it has apparently been regarded as a fermentation by-product.

Drews *et al.*[62] described recently a method for determination dependant upon purging by means of nitrogen the dimethyl sulphide from warm samples into potassium permanganate solution. The isolated dimethyl sulphide is oxidised to dimethyl sulphone, which is extracted and assayed by gas chromatography. The analysis by this method is time-consuming and a more direct procedure by injection of beer vapour would be advantageous. In addition this would eliminate the possible artefact formation which could arise at the high temperature, circa 65°C, used in the Drews sulphone technique.

The method to be described is based upon gas chromatography of beer vapour. The concentration of dimethyl sulphide is determined using a n-butanol internal standard. The reproducibility of the results obtained on a number of beers was reflected in the acceptable coefficient of variation (7.7%) from duplicate analysis of seventeen samples.

The identity of the dimethyl sulphide peak from beer was confirmed by comparison of retention data on two different stationary phases. Isolation and identification by infra-red analysis was precluded by the low amount of dimethyl sulphide normally present in the sample. Additional chemical confirmation of identity was obtained by comparing the reaction of the dimethyl sulphide from the sample, and from a similar sample containing added dimethyl sulphide, with a variety of reagents, viz. silver nitrate, hydrogen peroxide and mercury (II) chloride.

2.10 EXPERIMENTAL

2.11 Reagents

All reagents were of Analar quality unless otherwise specified.

(1) Ethanol solution:- absolute ethanol (35 ml) diluted to one litre with deionised water.

(2) n-Butanol standard:- n-butanol (200 mg) diluted to one litre with ethanol solution.

(3) Dimethyl sulphide standards in aqueous ethanol solution:- dimethyl sulphide (100 mg) was diluted to one litre and an aliquot (10 ml) of this solution was further diluted to 100 ml. Subsequent dilutions of this latter solution gave the necessary working standards. These should be freshly prepared each day. The concentration was checked by the method of Belcher et al. (see appendix B).

(4) Sodium chloride:- Solid.

2.12 Apparatus

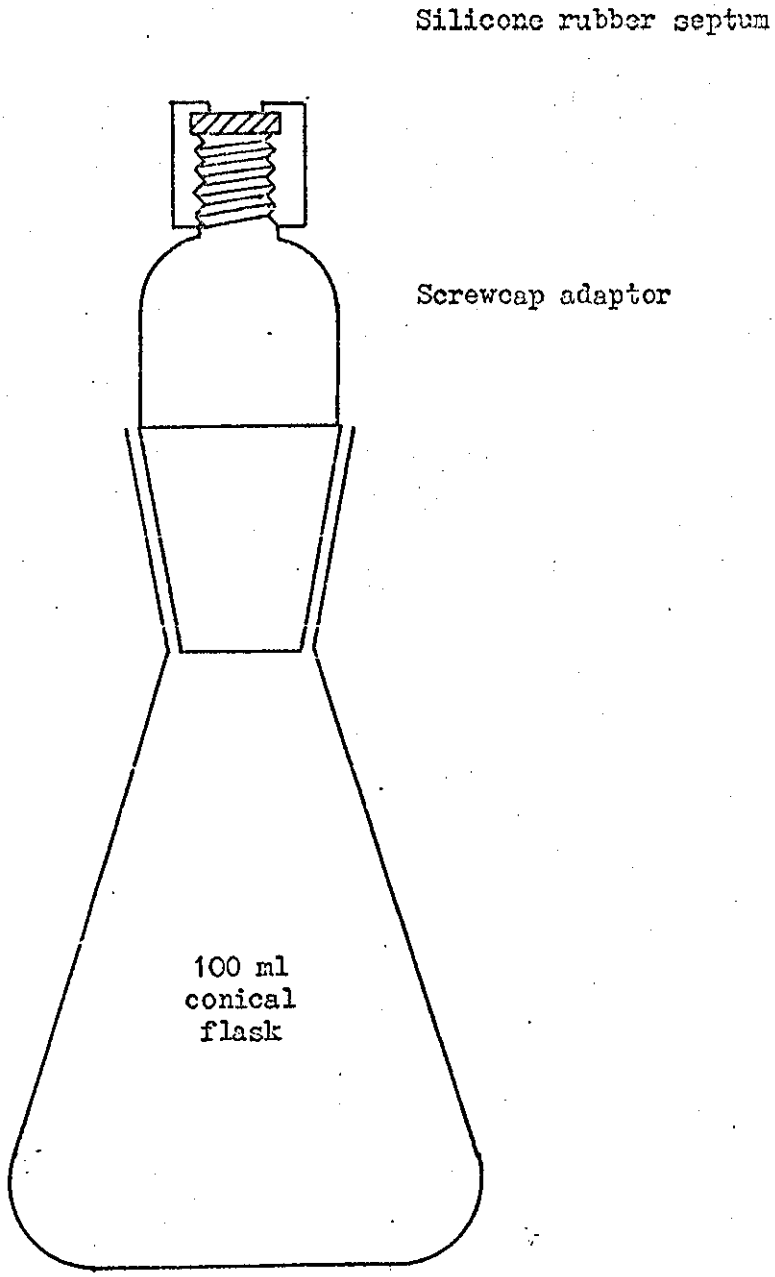
(1) F & M Model 810 research chromatograph with dual flame ionisation detectors. The operating conditions were as follows:-

Columns	- 10' x $\frac{1}{4}$" coiled stainless steel (18:8:1)
Solid support	- Embacel 60 - 80 mesh (May and Baker Ltd.)
Stationary phase	- 20% polyethylene glycol 1500 (Shell Chemicals Ltd.)
Column temperature	- Isothermal (50°C) for seven minutes then programmed at 60°C per minute to 130°C
Carrier gas	- Argon at 100 ml/min
Detector temperature	- 210°C
Injection temperature	- 110°C
Attenuation	- 1 x 4 changing to 1 x 16 after seven minutes
Recorder	- Honeywell "Electronik" potentiometric recorder
	Fullscale deflection 1 mv
	Response time 1 sec
	Chart speed 15 in per hour

(2) Water bath at 30°C (± 1°C)

(3) Sample flask for headspace analysis:- Conical flasks (100 ml) sealed with a B24 Quickfit and Quartz screwcap adaptor and silicone rubber septum, as shown in Fig. 2.1.

FIG. 2.1 - SAMPLE FLASK FOR HEADSPACE ANALYSIS



(4) Hypodermic syringe (20 ml), an Agla micrometer burette and a Hamilton gas syringe (10 ml).

2.13 Method

8 g sodium chloride and 0.1 g hydroxylammonium chloride were placed in a clean dry conical flask and immediately sealed with a Quickfit & Quartz screwcap adaptor and silicone rubber septum. 20 ml of beer (0°C) was added to the flask, by means of a hypodermic syringe followed by 0.5 ml n-butanol standard solution from a micrometer burette. The flask was placed in a water bath (30°C ± 1°C) for 90 minutes. A 10 ml sample of the vapour was withdrawn using a gas syringe and injected into the gas chromatograph.

The concentration of dimethyl sulphide in the sample was calculated from the heights of the dimethyl sulphide and n-butanol peaks (Fig. 2.2) using the relative response factor (F) for n-butanol and dimethyl sulphide as follows:-

$$\text{Concentration of dimethyl sulphide} = \frac{Cs}{Hs} \cdot \frac{Hx}{F}$$

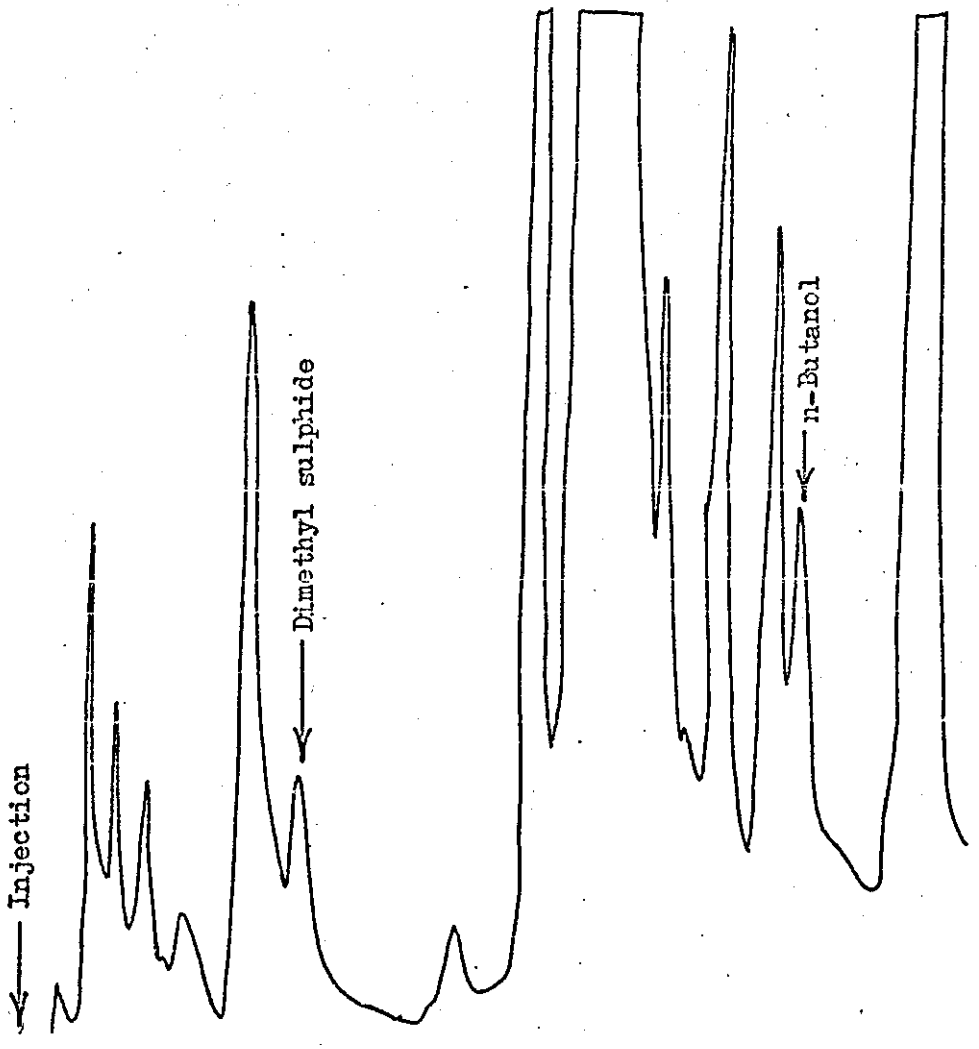
where Cs is the concentration of the standard in micrograms per litre and Hs, Hx are the respective peak heights of the standard and dimethyl sulphide.

A typical chromatogram is shown in Fig. 2.2. The dimethyl sulphide and n-butanol peaks are indicated.

2.14 Linearity of response

The linearity of the detector response is also shown by the data illustrated in Fig. 2.3.

FIG. 2.2 - TYPICAL GAS CHROMATOGRAM OF BEER RESPONSE



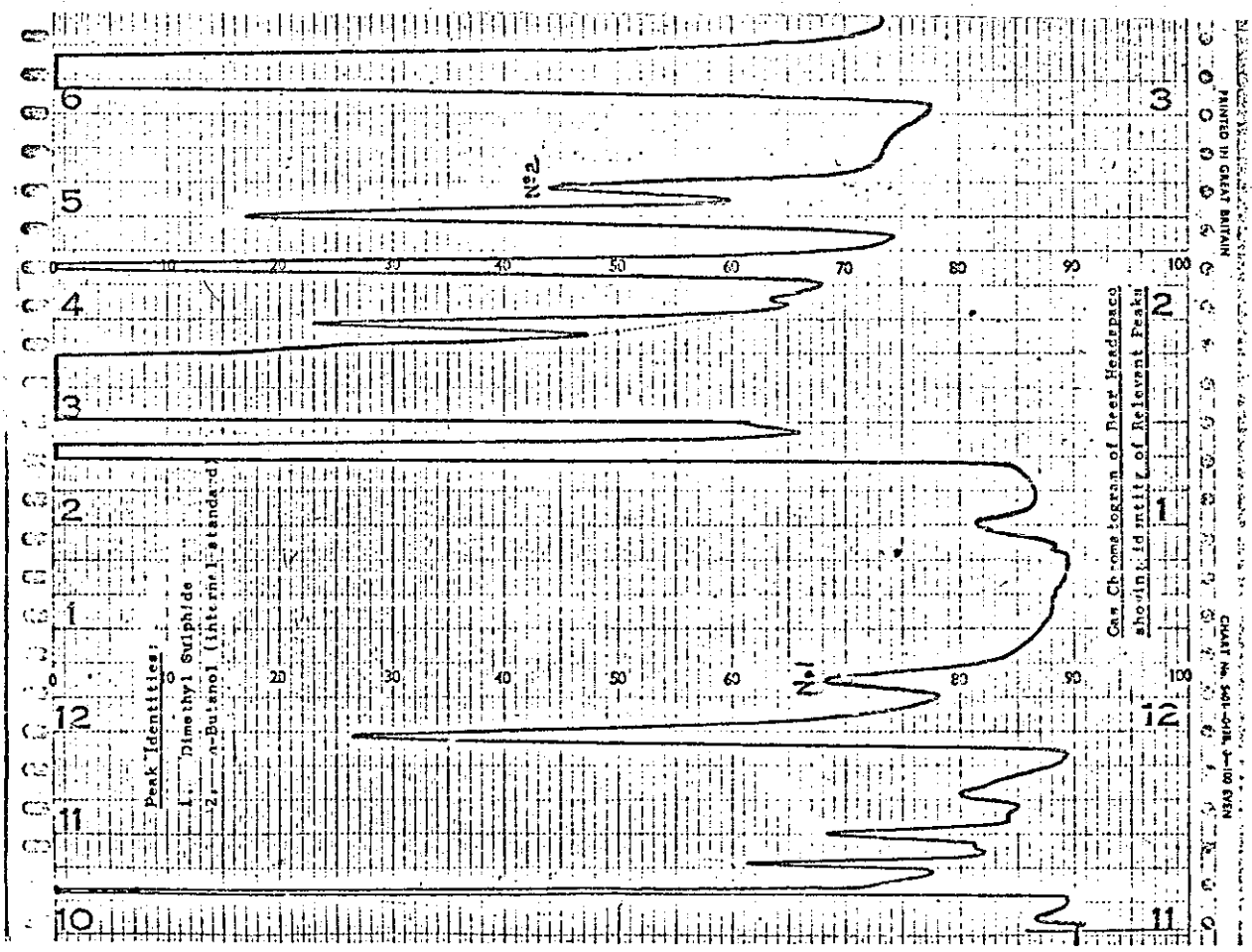
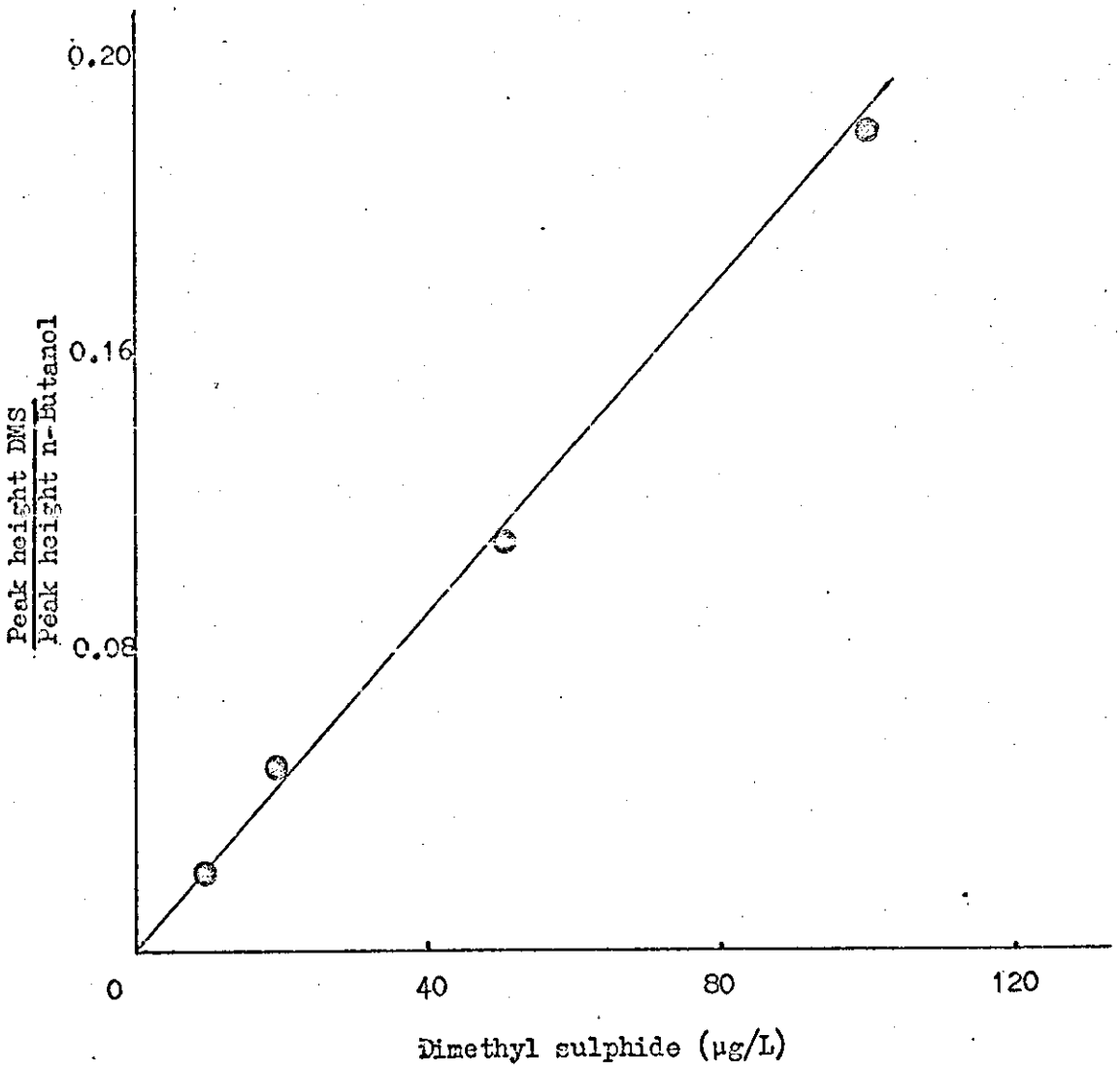

Peak Identities:
1. Dimethyl Sulphide
2. n-Butanol (Internal standard)
Gas Chromatogram of Beer Headspace
showing identity of Relevant Peaks
PRINTED IN GREAT BRITAIN

FIG. 2.3 - CALIBRATION CURVE SHOWING LINEARITY OF DETECTOR RESPONSE



2.20 RESULTS AND DISCUSSION

2.21 Determination of response factor

According to recommended gas chromatographic practice[63] the measurement of peak area provides the most exact relationship with concentration. However, in the present instance, it was found that measurement of peak height was adequate. For quantitative analyses it was necessary to optimise the gas chromatographic response and obtain the relationship between dimethyl sulphide and n-butanol responses to calculate the relative response factor (F).

i.e. $$F = \frac{Cs}{Hs} \cdot \frac{Hx}{Cx}$$

where Cs = concentration of standard (n-BUOH)

Cx = concentration of sample (DMS)

Hs = peak height of standard (n-BUOH)

Hx = peak height of sample (DMS)

The values for F obtained over the range of concentration 10 - 100 micrograms per litre dimethyl sulphide are illustrated in Table 2.1. These values show that this factor was constant over the given concentration range, the average value 11.50 was used for the calculation of dimethyl sulphide concentration in later experiments.

TABLE 2.1 - THE RELATIVE RESPONSE FACTOR FOR DIMETHYL SULPHIDE AND n-BUTANOL

	Dimethyl sulphide concentration (µg/L)			
	10	20	46	100
Response Factor	10.72	13.43	10.82	11.82
	13.19	11.26	10.97	10.56
	13.19	11.57	10.49	9.57
	13.21	13.16	12.49	9.87
	11.08	13.22	11.66	11.19
	12.68	10.87	11.19	10.78
	-	-	-	10.43
	-	-	-	10.69
	-	-	-	10.34
Average response factor = 11.50 Standard deviation = 1.16				

2.22 Identification of dimethyl sulphide peak

Before proceeding with the analysis of beers it was considered desirable to confirm the identity of the dimethyl sulphide found in beer. Comparison was made of

(1) retention data, and

(2) removal by chemical reaction of dimethyl sulphide from a sample of beer and from a similar sample to which dimethyl sulphide had been added.

(1) The retention volumes on two different stationary phases were measured. The effect of the adjacent peak, due to acetaldehyde, was measured by preparing a model system of aqueous ethanol, acetaldehyde and dimethyl sulphide. The results are given below in Table 2.2.

TABLE 2.2 - RETENTION VOLUME[+] FOR DIMETHYL SULPHIDE

Sample	Stationary phase	
	PEG 1500	* Tris CNEP
Control beer	268	303
Dimethyl sulphide (30 µg/L) + acetaldehyde (25 mg/L) in 3% v/v ethanol	277	306
Control beer + added dimethyl sulphide (30 µg/L)	268	300

* 1,2,3-Tris(2-cyanoethoxy) propane

[+] Retention volume = Carrier gas flow measured at the outlet (ml/min) x retention time (minutes)

(2) The reactions with silver nitrate, hydrogen peroxide and mercury (II) chloride of both the sample and authentic dimethyl sulphide were compared. In each instance the dimethyl sulphide peak disappeared when the headspace vapour was chromatographed.

The agreement found between the retention volumes and the reactions indicated that the two compounds were the same and the identity was therefore regarded as confirmed.

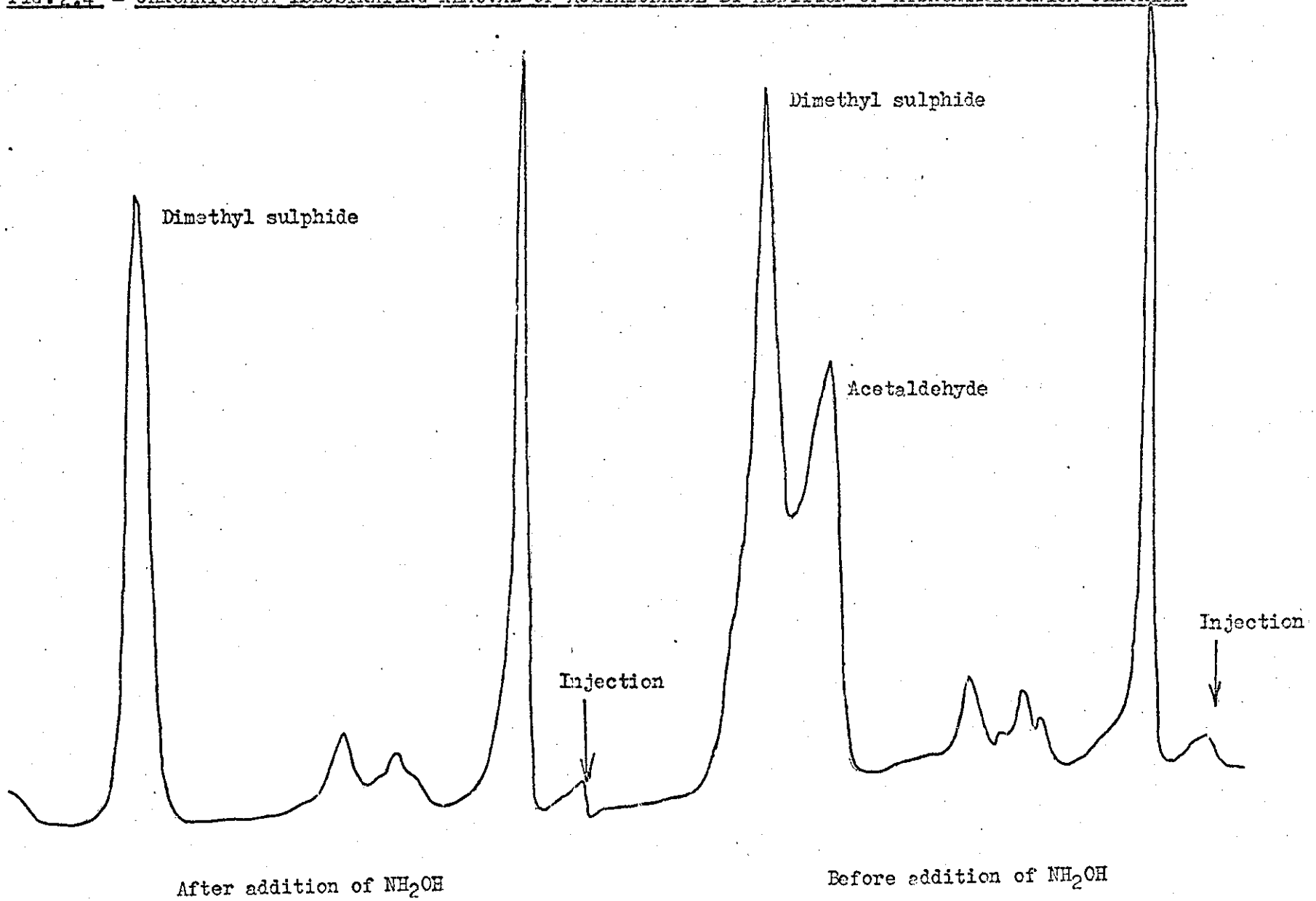
2.23 Sensitivity of method

The sensitivity of the method was taken to be the dimethyl sulphide concentration which was twice the standard deviation of the method. This is to some extent dependent on the noise level of the detector - so that a detection limit of twice the average base line noise was chosen as the lower limit of detection. The sensitivity was found to be approximately 3 micrograms per litre under normal working conditions.

2.24 Interference from acetaldehyde

Interference from acetaldehyde which was experienced in certain samples of beer was removed by adding a pre-column of FFAP (12" x $\frac{1}{4}$", 10% FFAP on Chromocorb W 80 - 100 mesh). Alternatively the addition of a small quantity of hydroxylammonium chloride (0.05 - 0.1 g) to the sample satisfactorily removed the interfering acetaldehyde. This modification is included in the recommended method. Chromatograms obtained before and after addition of hydroxylammonium chloride are illustrated in Figs. 2.4 (see also original chromatograms in Appendix C).

FIG. 2.4 - CHROMATOGRAM ILLUSTRATING REMOVAL OF ACETALDEHYDE BY ADDITION OF HYDROXYLAMMONIUM CHLORIDE



2.25 Dimethyl sulphide recovery from beer

The recovery of dimethyl sulphide added to beer, over a concentration range from 10 - 90 micrograms per litre, was examined and the results are given in Table 2.3. The upper limit of 90 micrograms was taken as three times the organoleptic threshold of 30 micrograms per litre of dimethyl sulphide reported previously by Harrison [2]. An average recovery of 99% was obtained with the chosen concentration range which is regarded as satisfactory.

TABLE 2.3 - RECOVERY OF DIMETHYL SULPHIDE (DMS) ADDED TO BEER

Sample	Added DMS (µg/L)	Total DMS found (µg/L)	Average DMS recovered (µg/L)	Recovery (%)
1	0	28		
	9	38 36 36	8.7	97
2	0	30		
	20	48 50 48	18.7	94
3	0	33		
	59	94 94 92	60.3	102
4	0	32		
	89	125 124 125	92	104

2.30 TYPICAL VALUES FOR THE DMS CONTENT OF ALE AND LAGER

A variety of bottled ales and lagers were analysed for dimethyl sulphide. The standard deviation (S_r) obtained from duplicate analyses of bottled beer reported in Table 2.4 was calculated from the formula:

$$S_r = \left[\frac{d^2}{2K}\right]^{\frac{1}{2}}$$

where each 'd' is the difference for a pair of duplicate results and 'K' is the number of samples[64]. This method of obtaining a measure of the reproducibility was chosen since it provides an average value for the standard deviation (2.12) and coefficient of variation (7.7%) more applicable to results when the method is used under routine conditions.

The levels of dimethyl sulphide found in the various beers analysed and given in Table 2.4 demonstrate two interesting features. Firstly, all the results obtained (0 - 35 µg/l) were considerably lower than the values obtained by Drews for Pilsner beers (120 - 140 µg/l). In the technique described by Drews the sample is heated briefly at 65°C and the volatiles expelled into potassium permanganate. It is possible that the differences between the present results and those of Drews may not only be due to the type of beer and brewing process but also to the effect of temperature on the sample.

TABLE 2.4 - DIMETHYL SULPHIDE (DMS) CONTENT OF BEER

Sample	Original Gravity* (° Sacch)	Dimethyl Sulphide (µg/l)	
Lager	33	43	49
	"	31	
	"	42	
	44	25	26
	"	18	19
	"	27	26
	"	25	24
	"	19	21
	"	33	25
Light Ale	32	4	5
	"	11	12
	"	10	13
Pale Ale	48	10	10
	"	0	0
	"	6	6
	"	5	7
	"	4	4
Brown Ale	32	13	11
	"	21	25
	"	29	30

2.40 EFFECT OF TEMPERATURE ON FORMATION OF DMS IN BEER

Formation of dimethyl sulphide, presumably by thermal decomposition of sulphur compounds, was demonstrated by Drews to occur on heating the sample. Constant results were obtained for vacuum distillation at each of the temperatures 75°C, 65°C and 55°C. No attempt was made to obtain results at temperatures lower than 55°C. The possibility of formation of dimethyl sulphide with increasing temperature within the range 20 - 80°C was determined by analysing a series of bottled lager beers (original gravity 1044). Duplicate samples were incubated at 20°C, 60°C and 80°C for one hour, cooled to room temperature (20°C) and re-equilibrated at 30°C and assayed by the technique previously described. A comparison of the results in Table 2.5 for the heated beer versus its control showed clearly that increased temperature had no effect on the analytical result, thus confirming the findings of Drews et al. for samples of beer not subjected to distillation.

TABLE 2.5 - EFFECT OF SAMPLE TEMPERATURE ON DIMETHYL SULPHIDE CONTENT OF BEER

Sample	Dimethyl sulphide (µg/l)	
Control (lager beer, OG = 44° Sacch	21	22
Control after 60 mins at 20°C	22	22
" " " " " 60°C	23	21
" " " " " 80°C	18	19

Differences in the dimethyl sulphide content of beers analysed by the present method and those quoted by Drews are considered to reflect the differences in British and Continental brewing practice. Nevertheless, since a figure of 30 micrograms per litre has been reported to be the flavour threshold of dimethyl sulphide, and since high concentrations of dimethyl sulphide usually impart an undesirable flavour, it is, in the author's opinion, difficult to reconcile the results obtained by Drews with normal production beer. Nevertheless, analysis of other Continental beers have shown considerably higher DMS values. Typical analyses are illustrated in Table 2.6. It is also evident from these results that the dimethyl sulphide is independent of original gravity (O.G.).

A further feature of interest arising from the results in Table 2.4 is the significant difference in level of dimethyl sulphide for the types of beer examined, namely ales and lager. Within the group analysed the average dimethyl sulphide content for ale made by top fermentation procedure is 11 µg/l whereas the average for lager made by a bottom fermentation production procedure is 24 µg/l.

TABLE 2.6 DIMETHYL SULPHIDE (DMS) CONTENT OF CONTINENTAL LAGER

Beers		DMS ($\mu g l^{-1}$)		OG (^{o}Sacch)
German Beer	DINKELACKER 'Export Dark'	44	36	52.1
	LÖWENBRÄU MUNCHEN (Light Special)	57	49	57.7
	HENNINGER (Diabetic Beer)	28	33	47.9
	MUNCH - GOLD Light BOCK - SPATEN Brewery	97	95	65.3
Tuborg Lager	ROYAL DENMARK BREW	67	69	67.6
Schlitz Beer	TEXAS U.S.A.	60	68	46.0
Pils Holsten	BRAUEREI (Diabetic Beer)	60	62	45.6
Holsten	PILSNER	93	101	45.1
Stella Artois (Belgium)	CONTINENTAL LAGER	81	80	36.7
Ringnes	PILSNER NORWAY	62	59	31.2
	HACKER BRÄU MUNICH	114	114	51.4
	KRONENBOURG 10.60°	90	89	60.6
	ORANJEBOOM DELUX	48		
Skol Pale Export		16		44
Carlsberg Pilsner		34		29
Harp Lager		34		33
Allsopps Lager TS 10/12		21, 17, 22		44

Note: O.G. = original gravity

This difference might be attributed to a number of factors. For example the different kilning used in the production of lager and ale malt provides different degrees of proteclysis and consequently a high yield of sulphur amino acids available to the yeast during lager fermentations. Another factor could be the metabolic differences between the yeast involved, namely S.cerevisiae and S.carlsbergensis. i.e. the yeast species used for ale and lager fermentations. A third possibility may be a purely physical effect due to the influence of carbon dioxide washout of volatiles during fermentation. This effect would be more efficient at the higher temperatures used in top fermentations (circa 20°C) than at the lower temperature (circa 7°C) used in bottom fermentation. Furthermore, prolonged lagering would not favour dimethyl sulphide removal by carbon dioxide.

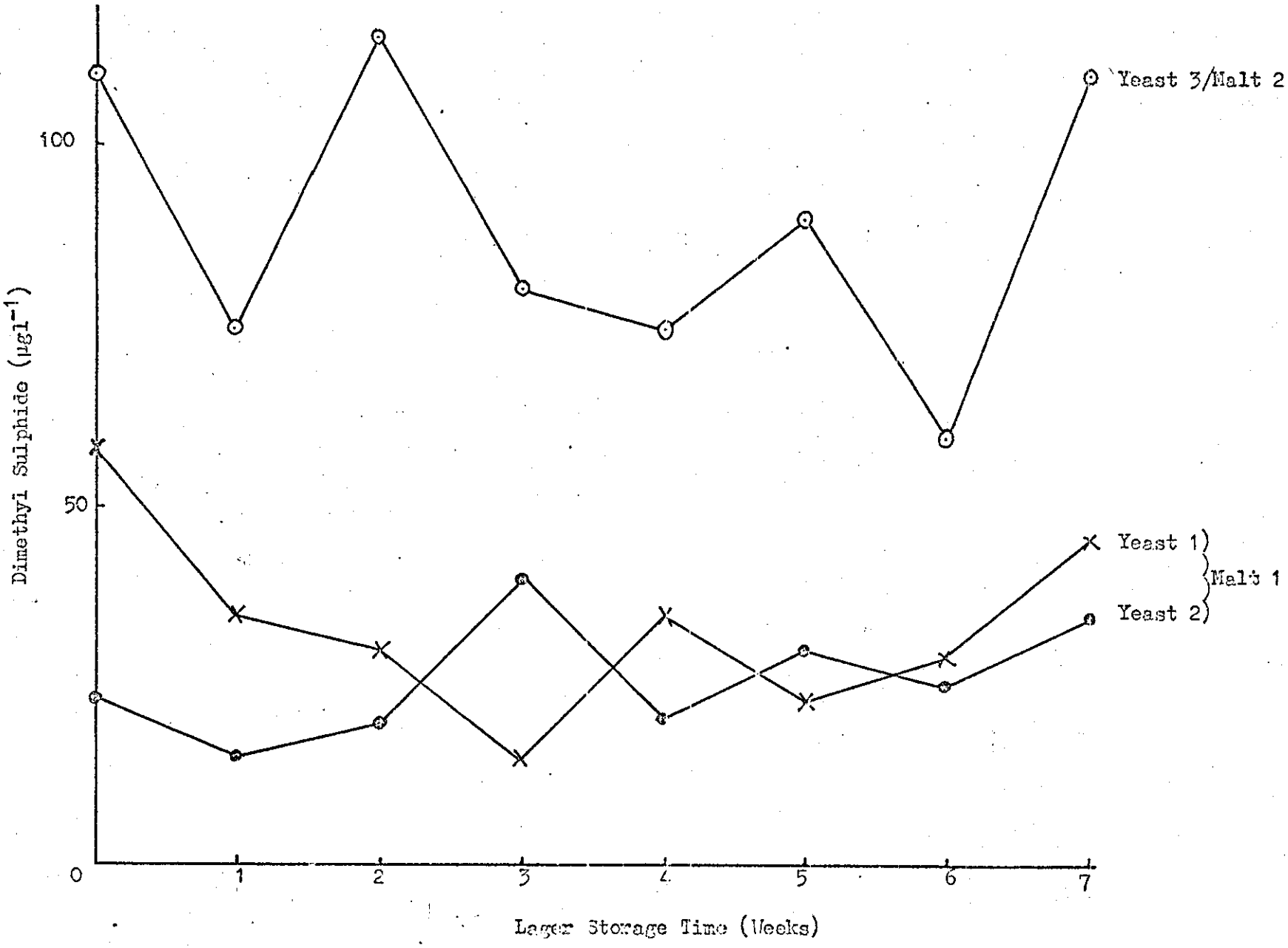
2.50 EFFECT OF LAGERING

As mentioned above the difference in the dimethyl sulphide contents of ale and lager could be due to the lagering process. This is of some technical interest since the reported odour threshold for dimethyl sulphide is close to the concentration of dimethyl sulphide found in British lager. It was therefore decided to establish if the dimethyl sulphide content of bottom fermented beer changed during the lagering period. Samples of lager were obtained at weekly intervals, yeast was removed, by centrifugation, and the samples analysed (Fig. 2.7).

Although the sample variance was high, no statistically significant trend in the dimethyl sulphide content during lagering could be established and this was also true for the fusel oil and ester content which were determined simultaneously by gas chromatography. These results raise doubt about the necessity for prolonged lagering and its effect on flavour. Lagering is considered necessary at the present time to remove haze forming material (e.g. protein-tannin complexes). The time required, at present approximately six weeks, could possibly be reduced by using malt which had been treated with formaldehyde or other stabilising additives.

Further process research on the efficiency of the lagering process is indicated since by reduction of the time involved considerable production economies could be realised.

FIG. 2.7 - EFFECT OF LAGERING ON DIMETHYL SULPHIDE



CHAPTER 3 - ELIMINATION OF NITRATE INTERFERENCE IN THE TRACE DETERMINATION OF SULPHATE AS HYDROGEN SULPHIDE

3.00 INTRODUCTION

During an investigation of non-volatile sulphur compounds in freeze dried beer it was necessary to determine the total sulphur content of chromatographic fractions which contained different amounts of nitrogenous material.

The Schöniger technique[65, 66] was used to convert organic sulphur to sulphate followed by reduction of the sulphate to hydrogen sulphide using the method of Gustafsson[45]. The hydrogen sulphide was determined colorimetrically after conversion to methylene blue. In preliminary studies of the assay of sulphur compounds in model systems results obtained within the range 0 - 40 micrograms sulphur were satisfactory. When, however, nitrogenous material was introduced the analytical precision and accuracy were impaired, with a bias to low recoveries. A similar effect had previously been noted by Gustafsson and by Johnson and Nishita[67] who suggested losses were due to the formation of volatile products which interfered with the formation of methylene blue.

When applied to synthetic sulphate/nitrate mixtures the low recovery of hydrogen sulphide in the reduction step became significant as the ratio of nitrogen:sulphur increased. It was found that previously described procedures for removal of nitrate ion with concentrated hydrochloric acid[67] or a formic

acid-hydrochloric acid mixture[68] gave variable results. These are considered to be due to the occasional presence of sulphur containing impurities in the reagents in addition to incomplete removal of the nitrate ion.

An alternative method based upon the differences in thermal stability of zinc sulphate and zinc nitrate[69] has been examined.[61] It was found that the addition of zinc acetate after Schöniger combustion followed by evaporation to dryness and ignition at 320°C gave reproducible and quantitative recoveries of sulphur in the subsequent reduction despite the presence of relatively high levels of nitrogenous material in the original samples.

3.10 EXPERIMENTAL

3.11 Reagents

All were of Analytical Reagent Grade unless otherwise state.

Hydrogen peroxide - (100 vol.)

Potassium permanganate - (4% w/v)

Glacial acetic acid

Zinc acetate absorption solution - 0.25 M zinc acetate in 0.1 M sodium acetate solution.

Zinc acetate - (0.1% w/v)

Iron (III) ammonium sulphate - 0.25 M Iron (III) ammonium sulphate in 0.5 M sulphuric acid.

Para-amino-N-dimethyl aniline - p-amino-dimethyl-aniline sulphate (0.93 gl^{-1}) in 3.5 M sulphuric acid.

Reducing solution - 2.5 g of sodium hypophosphite monohydrate dissolved in 25 ml glacial acetic acid and 100 ml (SG 1.7) hydriodic acid. This mixture was refluxed under nitrogen for one hour, cooled, stoppered, and stored in the dark. This solution is normally stable for one month. Any iodine produced can be removed by refluxing under nitrogen.

Nitrogen - "Oxygen-free" grade was further purified by passing consecutively through solutions of alkaline pyrogallol (10% w/v in 10% NaOH), and potassium permanganate (2.5% w/v) saturated with mercury (II) chloride.

Millipore membrane filters (cellulose acetate, 8 micron, 2.5 cm diameter).

3.12 Procedure

Oxidation

A 10 mg sample was weighed on a Millipore membrane and wrapped in a Whatman paper (No. 42, 5 cm diameter) and placed in the platinum basket of a 500 ml Schöniger combustion flask which contained 10 ml deionised water and 0.25 ml hydrogen peroxide. The Whatman paper was ignited and the basket plunged into the combustion flask which had been flushed previously with oxygen.

After combustion, the flask was shaken (circa 5 min) and allowed to stand for 5 min to ensure complete absorption of the combustion products. 3 ml glacial acetic acid was added, followed by 1 ml 0.1% w/v zinc acetate solution. The

contents were then quantitatively transferred to a reduction flask. 3 ml potassium permanganate solution was added to destroy excess hydrogen peroxide. The mixture was evaporated to dryness over a micro bunsen burner passing nitrogen through the solution at 150 ml min^{-1}. The flask and residue were ignited for one hour at 320°C in a muffle furnace.

Reduction

The flask and contents were cooled, 5 ml of the reducing solution were added and the solution refluxed gently for 15 min with nitrogen purging at 125 ml min^{-1}. The effluent gas stream was passed through a water trap to remove any impurities and hydrogen sulphide was absorbed in 10 ml zinc acetate absorption solution plus 70 ml deionised water contained in a 100 ml volumetric flask.

Colour development

After absorption of the hydrogen sulphide the volumetric flask was placed in a water bath at 20°C ± 1°C for ten minutes. 10 ml para-amino-N-dimethyl aniline reagent was carefully added to form a layer on the bottom of the flask. The contents were then gently mixed by swirling. Any zinc sulphide adhering to the inside of the delivery tube was dissolved by sucking some of the acidic mixture up into the tube. 2 ml of iron (III) ammonium sulphate reagent was added, the flask stoppered and the contents vigorously shaken for about thirty seconds. The

resultant solution was finally diluted to 100 ml with deionised water. After standing at 20°C for a further fifteen minutes the absorbance was measured at 667 nm in 1 cm cells against water as blank.

The reagent blank (including the Millipore filter and the filter paper) was determined and the absorbance deducted from that of the sample. Normal blank values lay within the range 0.004 to 0.009. The amount of hydrogen sulphide present was obtained either from a calibration graph or routinely by using an appropriate factor. Beer's law was obeyed over the range 0 - 40 µg hydrogen sulphide.

3.20 RESULTS AND DISCUSSION

Analysis of model systems containing methionine (Table 3.1) or potassium sulphate (Table 3.2) showed good recoveries of sulphur. When an excess of urea was added to methionine samples lower recoveries were observed (Table 3.1).

TABLE 3.1 - EFFECT OF NITROGEN AND ZINC ACETATE ON SULPHUR DETERMINATION IN METHIONINE

Sulphur added µg	Nitrogen added µg	Sulphur found µg		Sulphur found in presence of zinc acetate µg	
4.8	0	4.5	3.8	5.4	5.3
"	70	2.2	2.9	5.1	4.7
"	140	2.6	2.6	5.1	5.0
9.9	0	8.6	8.3	9.3	8.6
"	70	8.0	6.7	-	10.8
"	140	5.1	5.8	9.1	10.4
19.8	0	20.2	17.6	20.1	-
"	70	13.8	13.8	19.8	19.7
"	210	14.1	13.4	19.2	19.2
32.0	0	32.0	28.8	32.0	-
"	28	24.6	26.6	32.0	-
"	70	26.2	24.3	31.6	31.6
"	700	12.2		30.7	32.0

TABLE 3.2 - DETERMINATION OF SULPHUR ADDED AS POTASSIUM SULPHATE

Sulphur added µg	Sulphur found µg		Error µg
10	9.3	9.0	- 0.7
20	19.3	19.2	- 0.8
30	29.4	29.4	- 0.6

Confirmation that this effect was due to the presence of nitrate ion was shown by reduction of a known amount of sulphate in the presence of excess potassium nitrate (Table 3.3). The decrease in recovery with increase of nitrate ion concentration was evident. This interference has been eliminated by using the difference in thermal stability[69] of zinc sulphate and zinc nitrate.

TABLE 3.3 - THE EFFECT OF NITRATE ON THE REDUCTION OF SULPHATE

Nitrate ion added mg	Sulphur added µg	Sulphur found µg
-	50	48.1
0.5	50	31.9
1.0	50	16.3
5.0	50	3.7

By adding zinc acetate to the solution after combustion of the sample, evaporating to dryness, and igniting the residue it was possible to decompose the nitrate ion. When samples containing nitrate ion were treated identically except for the addition of zinc acetate, the results showed greater error (Tables 3.4 - 3.5). Although heating the residue may decompose nitrate ion it has been found that reliable removal takes place on heating in the presence of zinc acetate.

Typical results shown in Table 3.4 illustrate the improvement of the sulphur assay in the presence of large amounts of nitrogen (140 µg) as urea when zinc acetate was added. These confirm the

TABLE 3.4 - EFFECT OF NITROGEN (added as urea) AND ZINC ACETATE ON SULPHUR DETERMINATION

	S content added µg	S content added µg					
		Nitrogen added (micrograms)					
		0		70		140	
Zinc acetate present	5	5.0	5.0	5.3	4.4	4.7	5.2
	10	9.2	8.6	8.9	8.8	10.4	9.1
	20	18.4	18.3	19.6	18.4	19.8	19.7
Zinc acetate absent	5	4.2	5.4	5.0	4.3	1.3	0.7
	10	8.9	9.4	9.2	9.4	7.3	5.9
	20	19.1	18.9	18.4	18.7	13.2	11.4

TABLE 3.5 - DETERMINATION OF SULPHUR (10 µg) IN THE PRESENCE OF NITROGEN (200 µg) (added as urea prior to combustion)

Method	Sulphur found (µg)			Range	Mean
Boil with hydrochloric acid	4.0	7.8	3.3	4.5	5.0
Boil with formic acid/hydrochloric acid mixture	5.0	8.8	7.1	3.8	7.0
Ignition of combustion residue without addition of zinc acetate	5.1	9.1	6.6	4.0	7.4
	7.3	7.4	8.9		
No ignition of combustion residue and no addition of zinc acetate	5.1	5.8	7.3	2.2	6.1
	5.9	7.1	5.1		
Ignition of combustion residue after addition of zinc acetate	10.4	9.0	9.2	1.4	9.5
	9.1	10.4	).0		

previous observations (Table 3.1) and indicate a limiting nitrogen:sulphur ratio beyond which low results will be obtained.

Soep and Demoen[70] showed that nitrite and nitrate were formed when organic nitrogen compounds undergo oxidative combustion and the products are dissolved in water. After treatment with hydrogen peroxide and permanganate all the nitrogen is present as nitrate. Gustafsson and Johnson and Nishita ascribed the low sulphur assays obtained in their work to the existence of an unknown volatile compound arising from the interaction between nitrate ion and the hydrogen iodide present in the reducing solution.

However, in the present author's opinion the effect on sulphur recovery was due to the liberation of traces of iodine which were carried over into the zinc acetate which interfere in the formation of methylene blue. This latter effect, which is due to oxidation of hydrogen iodide, can be readily demonstrated by admitting air into the reducing solution before reduction.

Typical results, obtained routinely, when the recommended method was applied to biological material are given in Table 3.6. The nitrate interference is seen to vary from sample to sample and thus the zinc acetate addition and ignition is necessary to ensure nitrate removal.

TABLE 3.6 - SULPHUR CONTENT OF BEER RESIDUE

Sample	Sulphur content of freeze-dried residue (µg/mg)	
	No zinc acetate added	Zinc acetate added
1	3.3	5.0
	3.5	4.8
2	2.1	2.5
	2.1	-
3	6.3	6.7
	6.2	6.5
4	3.2	3.3
	3.0	-
5	5.1	5.8
	5.1	6.0
6	3.8	4.1
	4.0	4.3
7	1.7	1.8
	1.7	1.8
8	3.8	4.6
	3.7	4.5
Average replication error (Re)	0.08	0.11

Standard deviation $= \sqrt{\frac{d^2}{2n}} = 0.10$ where d = difference between duplicates.

CHAPTER 4 - THE DETERMINATION OF THE TOTAL VOLATILE ORGANO-SULPHUR COMPOUNDS IN BEER

4.00 INTRODUCTION

In the previous chapters (1 and 2) methods for the assay of hydrogen sulphide, and dimethyl sulphide in model systems and in beer have been described, and their application to ales and lagers discussed. However, it was considered possible that other volatile organo-sulphur compounds could be present, and remain undetected, since the methods were designed to be specific for hydrogen sulphide and dimethyl sulphide. Remaining trace amounts of sulphur volatiles would not be detected. Therefore it was decided to attempt to develop a procedure which would be applicable in routine analysis, for the estimation of the total volatile organo-sulphur content of beer. Agreement between the total volatile organo-sulphur content measured by the present method and the summation of values obtained for alkane thiols, measured by a modification of the method of Ikeya[71], and dimethyl sulphide, indicated that other organo-sulphur compounds not sought individually, or by class, were either absent or below the level of detection.

Although free hydrogen sulphide has not been found as a normal constituent of commercial quality beer, there are conditions when it could occur, for example in beer containing yeast or bacteria[74]. Removal of this potential interference

along with sulphur dioxide prior to analysis was therefore considered to be a pre-requisite, of any proposed method. Sulphur dioxide is added to beer during processing, as part of the "fining" procedure. In this procedure an isinglass solution, which contains sulphur dioxide as its preservative, is added as a clarifying agent. The sulphur dioxide content in beer is subject to a statutory limit of 70 mgl^{-1} (75). Although the sulphur dioxide content of beer seldom exceeds 10 - 15 mgl^{-1} (as found in typical routine quality control assays) this is approximately a thousandfold in excess of the volatile organo-sulphur compounds normally found. The selective and quantitative removal of this compound from analysis samples was therefore essential.

Previous work, in this laboratory, had shown that both hydrogen sulphide and sulphur dioxide could be selectively removed from a gas stream, using a trap containing zinc acetate in a sodium acetate buffer. This simple separation procedure was adopted in this instance. To estimate the volatile organo-sulphur content of beer it was decided to utilise the nitrogen gas purging and assay the sulphur content of the resultant gas stream, after the selective removal of hydrogen sulphide and sulphur dioxide.

4.10 EXPERIMENTAL

4.11 Reagents

All reagents, unless otherwise stated, were of Analytical reagent grade, and solutions were prepared using deionised water.

The following reagents, solids and solutions were required in addition to those for the determination of hydrogen sulphide given in Chapter 1.

(a) Ammonium chloride

(b) Ascorbic Acid: To comply with the British Pharmacopoeia (1968)

(c) Ethylene diamine tetracetic acid - disodium salt

(d) Potassium permanaganate (4% w/v)

(e) Hydrogen peroxide (100 vol.)

(f) Potassium hydrogen carbonate (10% w/v)

(g) Oxygen

(h) Zinc acetate (0.25 M) solution in sodium acetate (0.10 M) solution

4.12 Apparatus

The apparatus used was as described for the determination of sulphate by reduction to hydrogen sulphide, assayed as methylene blue, (Chapters 1 and 5) plus the following:

(a) Round-bottomed, 3-necked flask (2 litre)

(b) Absorption tube (Arnold type) fitted with B12 joints

(c) Silica combustion tube (45 cm x 1 cm) packed with quartz wool

(d) Tube furnace, Baird and Tatlock Ltd. (Model No. 191)

(e) To avoid contamination and to ensure that the apparatus was leak-proof all joints were ground glass (B10) and lubricated with water

4.13 Procedure

Isolation procedure:

The apparatus was assembled as shown in Figure 4.1. 10 ml of potassium bicarbonate solution and 0.5 ml hydrogen peroxide solution were added to the flask (C), to ensure quantitative absorption of sulphur dioxide, and 10 ml zinc acetate solution to the Arnold absorption tube (B). The apparatus was purged with nitrogen prior to use.

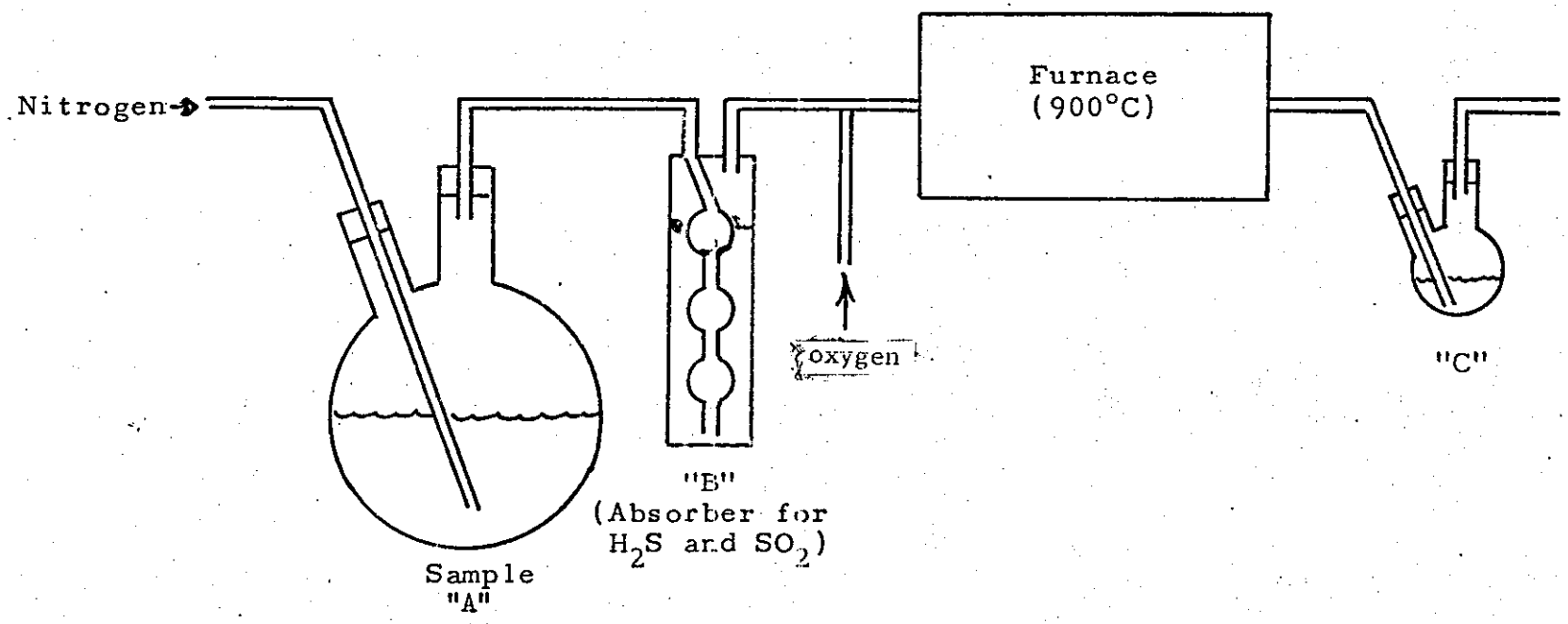
300 g Ammonium chloride, 1 g disodium-ethylene diamine tetra-acetate and 5 g ascorbic acid were added to 1 litre of beer or other sample, contained in the round-bottomed flask (A).

Nitrogen gas at a rate of 30 ml min^{-1} was passed through the sample which was held at 40°C in a water bath. Oxygen at a rate of 70 ml min^{-1} was mixed with the nitrogen stream prior to being passed through the heated (900°C) silica combustion tube.

After four hours, flask "C" was disconnected and 2 ml glacial acetic acid and 0.5 ml potassium permanganate solution added to its contents. The solution was evaporated to dryness under a stream of nitrogen whilst heating over a micro-bunsen.

The sulphur content was determined by reduction of the sulphate to hydrogen sulphide (Chapter 3) followed by estimation of the hydrogen sulphide as described in Chapter 1.

FIG. 4.1 APPARATUS FOR THE DETERMINATION OF VOLATILE ORGANO-SULPHUR COMPOUNDS IN BEER



4.20 RESULTS AND DISCUSSION

4.21 Optimisation of Combustion Conditions

A known volume of a standard dimethyl sulphide solution containing the equivalent of 30 µg S was added to the sample flask, which contained one litre of aqueous ethanol (4% v/v) and 300 g ammonium chloride. This mixture was then purged with nitrogen at 40°C, as described in section 4.13. The furnace temperature, total gas flow rate through combustion chamber and oxygen to nitrogen ratio were altered and the analysis of aliquots of the dimethyl sulphide standard solution repeated after each systematic alteration in the gas flow rate and furnace temperature.

The results in Table 4.1 illustrate the recovery of dimethyl sulphide (as sulphur) obtained under the different conditions examined.

TABLE 4.1 - PERCENTAGE RECOVERY OF DIMETHYL SULPHIDE (DMS) UNDER VARIOUS CONDITIONS

		Percentage Recovery of DMS			
Furnace temperature		500°C		900°C	
Total gas flow rate through furnace	ml min^{-1}	50	100	50	100
Oxygen:Nitrogen ratio	2:1	20	35	89	102
	1:1	15	25	85	92
	1:2	20	27	84	93

From these results it was evident that the best combustion conditions were as follows:

Furnace temperature	900°C
Total gas flow rate	100 ml min^{-1}
Oxygen:Nitrogen ratio	2.5:1

(i.e. 75 ml min^{-1} O_2:30 ml min^{-1} N_2)

These conditions were therefore adopted and used to analyse a further series of standards, namely alkane thiol (C_1 - C_5) viz. methanethiol, ethanethiol, propanethiol, butanethiol, isopentanethiol and dimethyl sulphide.

4.22 Linearity and Accuracy of Method

Standard aqueous ethanolic solutions of the C_1 - C_5 alkane thiols and dimethyl sulphide were prepared and standardised by the methods of Siggia[72] and Belcher et al.[73] (see appendices A and B).

Aliquots of each standard solution covering the range 5 - 25 µg sulphur were added to a model system, which was one litre of 4% aqueous ethanol containing 300 g ammonium chloride. These solutions were then analysed by the procedure described using the optimum flow and combustion conditions.

The results show that the good recoveries were obtained with each standard examined, as demonstrated by data in Tables 4.2 and 4.3 and shown diagrammatically in Figures 4.2 and 4.3.

TABLE 4.2 - RECOVERIES OF DIMETHYL SULPHIDE FROM A MODEL SYSTEM

Dimethyl sulphide (µg S)	
Added	Recovered
5.9	6.4
9.8	9.8
11.5	10.8
15.2	14.2
16.7	15.6
18.4	17.4
23.4	19.8
24.1	21.4
30.1	28.5
35.3	31.7
36.7	34.2

TABLE 4.3 - RECOVERIES OF ALKANE THIOLS FROM A MODEL SYSTEM

Alkane thiol (µg S)	
Added	Recovered
3.2	3.2
6.1	6.9
12.0	12.3
18.7	17.1
21.9	20.3
32.7	32.4

FIG. 4.2 CONVERSION OF DIMETHYLSULPHIDE TO HYDROGEN SULPHIDE

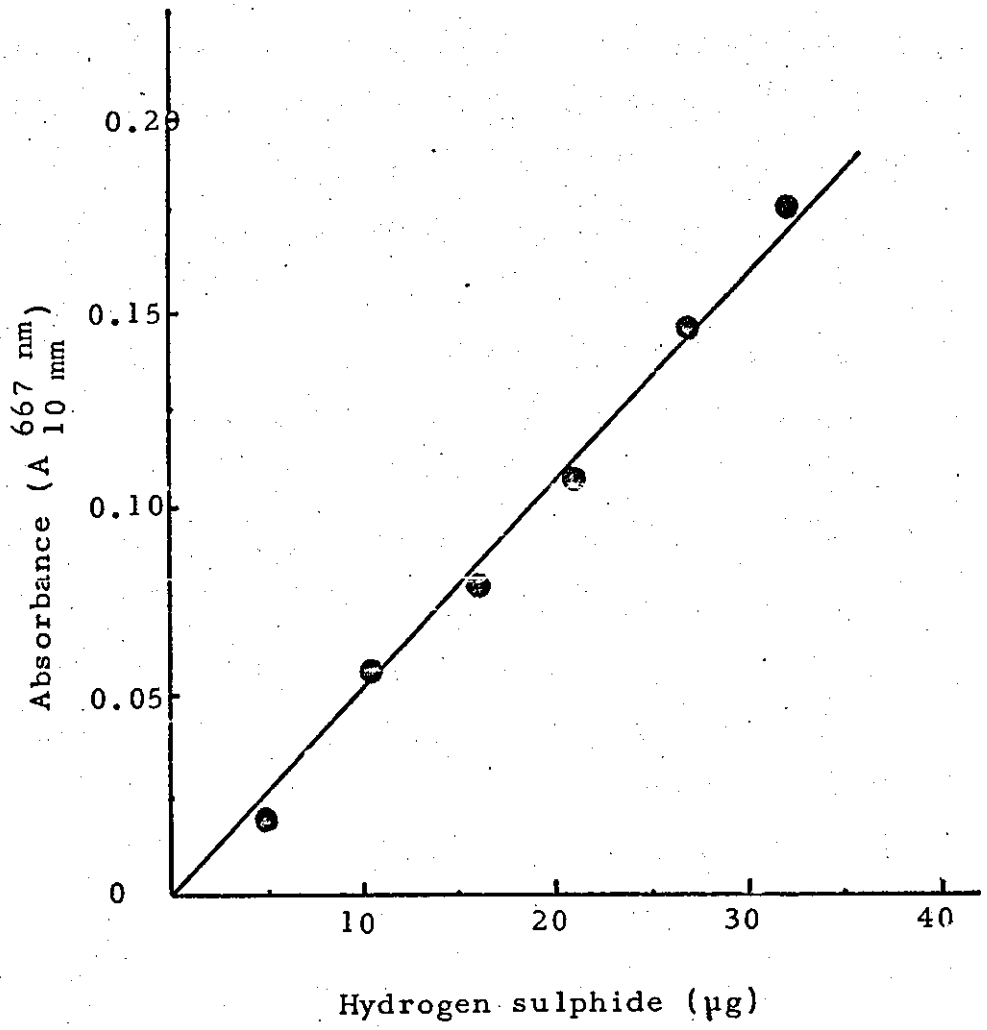
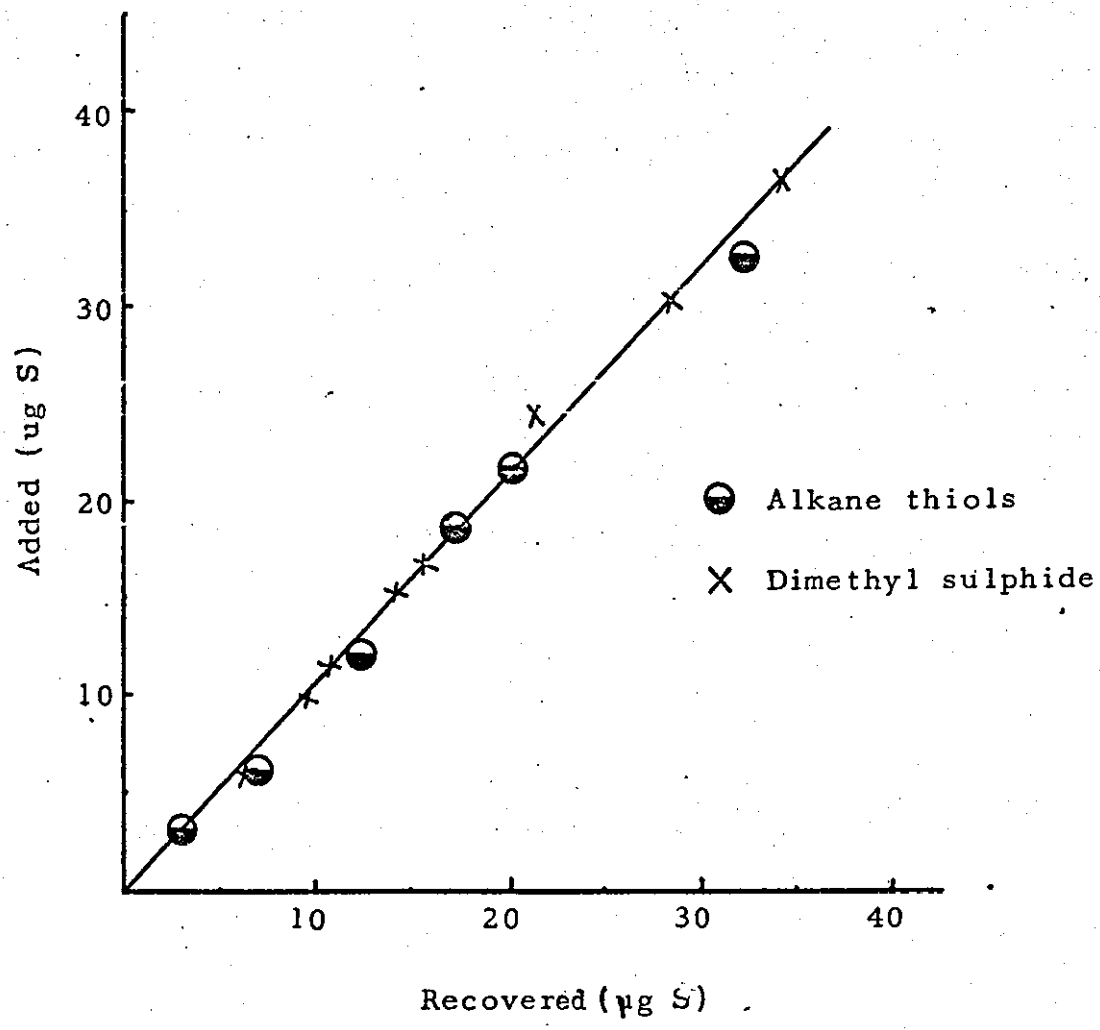


FIG. 4.3 - CORRELATION OF ADDED AND FOUND VALUES FOR TVOS DETERMINATION

(TVOS = Total volatile organic sulphur)



4.23 COMPARISON OF THE TOTAL VOLATILE ORGANO-SULPHUR (TVOS) METHOD AND THE SUM OF INDIVIDUAL ASSAY OF DIMETHYL SULPHIDE (DMS) AND ETHANETHIOL (ETSH)

Solutions of pure ethanethiol and pure dimethyl sulphide were prepared in 8% aqueous ethanol and standardised.

Aliquots of these solutions contained respectively known amounts, approximately 5 µg S as ethanethiol and 10 µg S as dimethyl sulphide were added to 5 litres of aqueous ethanol, (4% v/v). This solution was then assayed for TVOS, DMS and EtSH by the procedure already described.

The results are shown in Table 4.4. These illustrate a correlation between the assay of individual components and the total volatile sulphur estimation.

TABLE 4.4 - SULPHUR BALANCE BETWEEN TOTAL VOLATILE ORGANO-SULPHUR ASSAY AND ALKANE THIOLS AND DIMETHYL SULPHIDE

Added			Found		
DMS	EtSH	TVOS	DMS	EtSH	TVOS
expressed as S µg l^{-1}					
11.4	8.4	19.8	10.4	7.1	18.0
11.2	7.7	18.9	11.0	6.7	16.7
11.8	7.2	19.0	13.0	6.7	17.7

Similar experiments were made using beer in place of a model system.

TABLE 4.5 - RECOVERY OF ORGANIC SULPHUR IN THE PRESENCE OF HYDROGEN SULPHIDE (10 P.P.B.) AND SULPHUR DIOXIDE (20 P.P.M.)

Organic Sulphur (µg S)	
Added	Found
26.1	24.1
27.0	26.4
23.9	23.4

Although the concentrations of hydrogen sulphide and sulphur dioxide initially chosen were the maxima likely to be encountered in bottled beer, the recoveries of total volatile organic sulphur in the presence of other concentrations of hydrogen sulphide and sulphur dioxide were determined (Table 4.6) for the sake of completeness.

TABLE 4.6 - RECOVERY OF VOLATILE ORGANIC SULPHUR IN THE PRESENCE OF HYDROGEN SULPHIDE AND SULPHUR DIOXIDE

Added					Found
SO_2	H_2S	RSH	DMS	TVOS	TVOS
mg	µg	as µg S			as µg S
20	30	7.2	3.5	10.7	9.6
20	30	7.1	6.7	13.8	12.2
20	30	14.3	15.1	29.2	26.6
40	60	5.6	1.7	7.3	7.1

SO_2 = sulphur dioxide

H_2S = hydrogen sulphide

RSH = alkanethiol

DMS = dimethyl sulphide

These results have been examined using simple statistical procedures.

Average "D" = 0.55 Bias = + 0.4

Relative bias = Bias $\pm$ standard deviation of bias

= + 0.55 $\pm$ 0.39

Variance ratio "F" $= (n-2)/\left(\frac{BC}{A_2} - 1\right) = 10/\left(\frac{2.28 \times 62.43}{(0.99)^2} - 1\right)$

$$= \frac{10}{\frac{142.34}{0.980}} - 1$$

$$= \frac{10}{145.244} = 0.068 - 1$$

$$\therefore F = 0.932$$

This value for F is much less than the 5% value of F for $Q_1 = 1$ and $Q_2 = n - 2$ so that there is no reason to consider that a significant difference exists between the two sets of results. It is thus reasonable to assume that the volatile organo-sulphur content is derived mainly from dimethyl sulphide and alkane thiols in normal beer.

Because of this conclusion it was considered of interest to analyse additional samples of beer to test:-

(a) The effect of pasteurisation on the volatile sulphur content.

(b) If so called "sulphury" beer (as described by brewery taste panels) contains greater than the normal amounts of volatile organo-sulphur compounds.

4.24 THE TOTAL VOLATILE ORGANO-SULPHUR CONTENT OF BEER

Samples of normal production ale and lager were assayed for total volatile organo-sulphur using the method described herein. The samples were also analysed for alkane thiols and dimethyl sulphide by the procedures described earlier. The results (Table 4.7) again illustrate a good correlation between the total sulphur assay and sum of the assays of the individual compounds.

TABLE 4.7 - TOTAL VOLATILE ORGANO-SULPHUR CONTENT OF BEER

(all results expressed as µg sulphur per litre of sample)

Sample	DMS	RSH	DMS + RSH "A"	TVOS "B"	Difference "D" D = B - A
1	2.3	3.2	5.5	5.3	- 0.2
2	2.0	2.0	4.0	4.9	+ 0.9
3	2.4	3.6	6.0	6.6	+ 0.6
4	2.1	4.1	6.2	6.1	- 0.1
5	2.0	3.6	5.6	5.8	+ 0.2
6	1.9	4.0	5.9	5.0	- 0.9
7	2.4	5.4	7.8	7.4	- 0.4
8	3.1	4.1	7.2	6.1	- 0.9
9	2.2	2.6	4.8	4.8	- 0
10	3.6	1.5	5.1	6.5	+ 1.4
11	1.8	2.7	4.5	4.2	- 0.3
12	2.0	1.6	3.6	2.6	- 1.0

TABLE 4.8 - EFFECT OF PASTEURISATION ON VOLATILE SULPHUR CONTENT OF LAGER

(All samples had an original gravity of 8.2°P)

Sample	Dimethyl sulphide		Alkanethiol		Total volatile sulphur	
	µg S per litre					
1. UP	9	10	4	5	14	14
P	8	9	4	5	11	14
2. UP	11	11	3	4	13	14
P	9	9	3	3	12	12
3. UP	9	9	4	4	11	12
P	8	8	5	5	12	12
4. UP	17	17	1	3	18	16
P	14	14	2	2	17	16
5. UP	2	3	5	6	9	10
P	3	5	6		9	
6. UP	9	9	4	3	11	12
P	8	8	5	6	12	11
7. UP	18	18	3	2	19	17
P	15	15	1	3	18	17

UP = unpasteurised sample

P = pasteurised sample

4.25 THE EFFECT OF PASTEURISATION ON TVOS CONTENT OF LAGER

The effect of pasteurisation on lager is sometimes associated with the occurrence of a distinctive change in odour. To assess whether this off-flavour could be due to a change in the concentration of sulphur volatiles, samples of lager were analysed before and after pasteurisation. These samples, taken from different brews, were removed from the production bottling line before and after pasteurisation. These beer samples were analysed for TVOS, DMS and RSH. The results are reported in Table 4.8 and show that pasteurisation had no detectable effect on the volatile sulphur content within the variability of the method, viz. using the relationship $\sqrt{\frac{d^2}{2n}}$ the standard deviation of the replication error (Sr) of the TVOS method was found to be 0.960 $\mu g l^{-1}$. 95% Confidence limits for TVOS analysis are 1.920 $\mu g\, l^{-1}$.

Whether the analytical methods were too insensitive to detect the small changes in concentration which affect odour cannot be decided, particularly since many non-sulphur compounds present also affect odour. However since the odour theshold of many sulphur compounds is low (see Introduction) it is possible that very small changes in concentration (< 1 $\mu g\ l^{-1}$) can alter the aroma of beer.

4.26 THE ANALYSIS OF "SULPHURY" BEER

In the production of beer, taste evaluation by the brewery taste panel is used as part of the normal quality control system. In many instances beer is described as possessing a "sulphury character", "dirty nose", "burned aroma". Often this aroma or character can be associated with beer which has been allowed to remain in contact with yeast for a prolonged period after the end of fermentation. Since the presence of unpleasant aromas can affect the acceptability of the product such beers have to be blended with other beer to produce an acceptable product and thus avoid total wastage of a particular brew. This operation, along with a possible increase in storage time of beer, obviously affects the economics of production output.

Although the taste panel often ascribes such character to the presence of a sulphur compound the true identity of the component(s) responsible is, at present, unknown. Samples of beer which were regarded as "sulphury" were therefore analysed and the results compared with apparently normal control samples. The results obtained are given in Table 4.9.

TABLE 4.9 - ANALYSIS OF "SULPHURY" BEERS AND LAGER

Sample	Original gravity	DMS	RSH	TVOS
	°P	μg Sl^{-1}		
Ale 1	10	0.6	3.7	4.3
2	"	0.8	3.0	6.1
3	"	ND	2.6	3.6
4	"	ND	2.0	3.3
5	"	2.3	3.2	5.3
6	"	1.8	2.7	4.2
Ale Control (Average of 30 samples)	"	1.7	3.5	4.9
Lager 7	11	29.0	1.6	33.0
Control 8	"	27.0	2.5	28.0

From these results no analytical evidence exists which correlates the incidence of "sulphury" character with volatile organo-sulphur content. It should be remembered that taste panel assessmentsare subjective, unless trained to recognise specific odours as in the perfumery industry. Therefore in the author's opinion the so-called "sulphury" aromas may arise from two effects namely

(a) Synergistic effects between individual components, one of which may be sulphur containing.

(b) An as yet unrecognised component which occurs in beer, perhaps as a result of yeast autolysis.

Attempts were made to isolate active components by purging 5 litre samples of the sulphury beer with nitrogen and condensing the gaseous effluent in liquid nitrogen. The isolates obtained had similar odour characteristics to the original beers. Separation of the isolated material on a gas chromatograph column etc., using a stream splitter at the detector was attempted. One portion of the gas stream was fed to the detector and the other was examined using the olfactory organ in an attempt to identify the component responsible for characteristic aroma. This simple approach to a highly complex problem, was unsuccessful and this aspect of the project was discontinued.

GENERAL CONCLUSIONS

In the foregoing chapers the development, modification and application of simple analytical methods for the determination of trace quantities of various sulphur compounds in beer have been described and discussed in detail. The major objective of this work being to develop simple and robust methods which could be applied in routine quality control analysis.

From the results obtained when these methods were used to analyse a variety of beer samples it has become evident that the incidence of so-called "sulphury" or "sulphidic" flavours in beer may not be due, as once thought, to the presence of hydrogen sulphide or thiols. However, two factors of possible importance to the brewing industry from this study are considered to be:-

i. The role of bound hydrogen sulphide and its relationship with beer stability in terms of flavour and clarity.

ii. The incidence of quantities of dimethyl sulphide in lagers which generally exceed the accepted flavour threshold values.

The general hypothesis concerning the role of bound hydrogen sulphide and the possible nature of its origin has been propounded in the chapter dealing with hydrogen sulphide. However, it should be mentioned at this stage that a more detailed biochemical investigation and elucidation of this problem may produce results which will provide not only a clearer understanding of beer stability but also information on the nature of the brewing process and its raw materials. In the author's opinion the investigation

of the bound hydrogen sulphide precusor warrants further investigation because of its apparent importance to flavour stability.

With regard to the volatile organo-sulphur derivatives which have been found in beer, i.e. dimethyl sulphide and alkanethiols, it has become evident that thiols do not appear to be of significance in beer flavour, based upon an analytical assessment of their concentration. However, this does not preclude the importance of such compounds, existing below the detection limit, and forming flavourful odours by means of a synergistic reaction. The proof of such reactions would be difficult to obtain without recourse to ultra sensitive analytical methods.

Dimethyl sulphide has now been shown to be an important constituent of lager and at moderate concentration ($\not<$ 70 ppb) may provide some of the flavour characteristics of that product. Since flavour is ultimately a subjective sensation, no hard line can be drawn between desirable and undesirable flavour. Therefor, the finding that lager has a higher dimethyl sulphide content than ale should not be interpreted as implying the existence of an undesirable off flavour in lager. Nevertheless the information on the mode of production of this compound could be of critical importance in process control. Whether the incidence of this compound is due to variation in the amino acid constituents of wort (and hence related to malt quality) which affect yeast metabolism, differences in yeast strain, or, as been

recently suggested, to wild yeast or bacterial contamination, is as yet unknown.

Evidence for the affect of bacterial contamination of beer by Zymomonas anerobia has been demonstrated by the author (74). It is considered that further studies should be made of the role of yeast and micro-organisms on the evaluation of sulphur volatiles.

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APPENDIX A

THE STANDARDISATION OF ALKANETHIOL SOLUTIONS BY POTENTIOMETRIC TITRATION

Reagents

Aq silver nitrate (0.01 N)

Aq sodium acetate (0.3 N)

Alkanethiol solution - Alkanethiol (equivalent to 50 mg SH^{1}) is weighed into a tared conical flask (100 ml) containing methanol (35 ml). This solution is then diluted to standard volume (1 litre) with aqueous methanol (50% v/v). An aliquot (10 ml) of this solution is diluted to standard volume (100 ml) with water and the thiol content determined.

Apparatus

E.I.L. pH meter - Vibron type 39A

Calomel reference electrode

Silver indicator electrode

Magnetic stirrer

Procedure

The electrode system is immersed in the aqueous sodium acetate (50 ml) contained in a beaker (250 ml) and the aqueous alkanethiol solution (95 ml) added. The remaining solution (5 ml) is used for standardisation of the method under investigation.

Standard silver nitrate solution is added in 0.1 ml increments to the above mixture with continuous stirring and, after five seconds, the EMF value of the cell is recorded.

A sudden drop of the potential of the silver electrode to the less negative values occurs at the end point.

It was found that in order to obtain good curves the silver electrode must be cleanned with a proprietory silver cleaner, and polished bright with paper tissue prior to use.

A titration curve is constructed by plotting the EMF value of the cell versus volume of 0.01 N silver nitrate solution added (Table A.1, Fig. A.1). The content of alkanethiol is then calculated as follows

Calculation

From the graph, the end point ≡ 1.36 ml 0.01 N $AgNO_3$

Since one equivalent of $AgNO_3$ ≡ one equivalent of SH'

$\therefore$ one ml 0.02 N $AgNO_3$ ≡ $\frac{\text{mol wt SH' gram}}{100 \times 1000}$

Alkanethiol (as SH') equivalent to one ml of 0.01 N $aGNO_3$

≡ $\frac{33.07}{100 \times 1000}$ gram SH'

≡ 330.7 µg SH'

$\therefore$ 1.36 ml 0.01 N $AgNO_3$ ≡ 1.36 x 330.7 µg SH'

≡ 449.8 µg SH'/95 ml solution

$\therefore$ Thiol content per 5 ml of solution ≡ $\frac{449.8}{19}$ µg

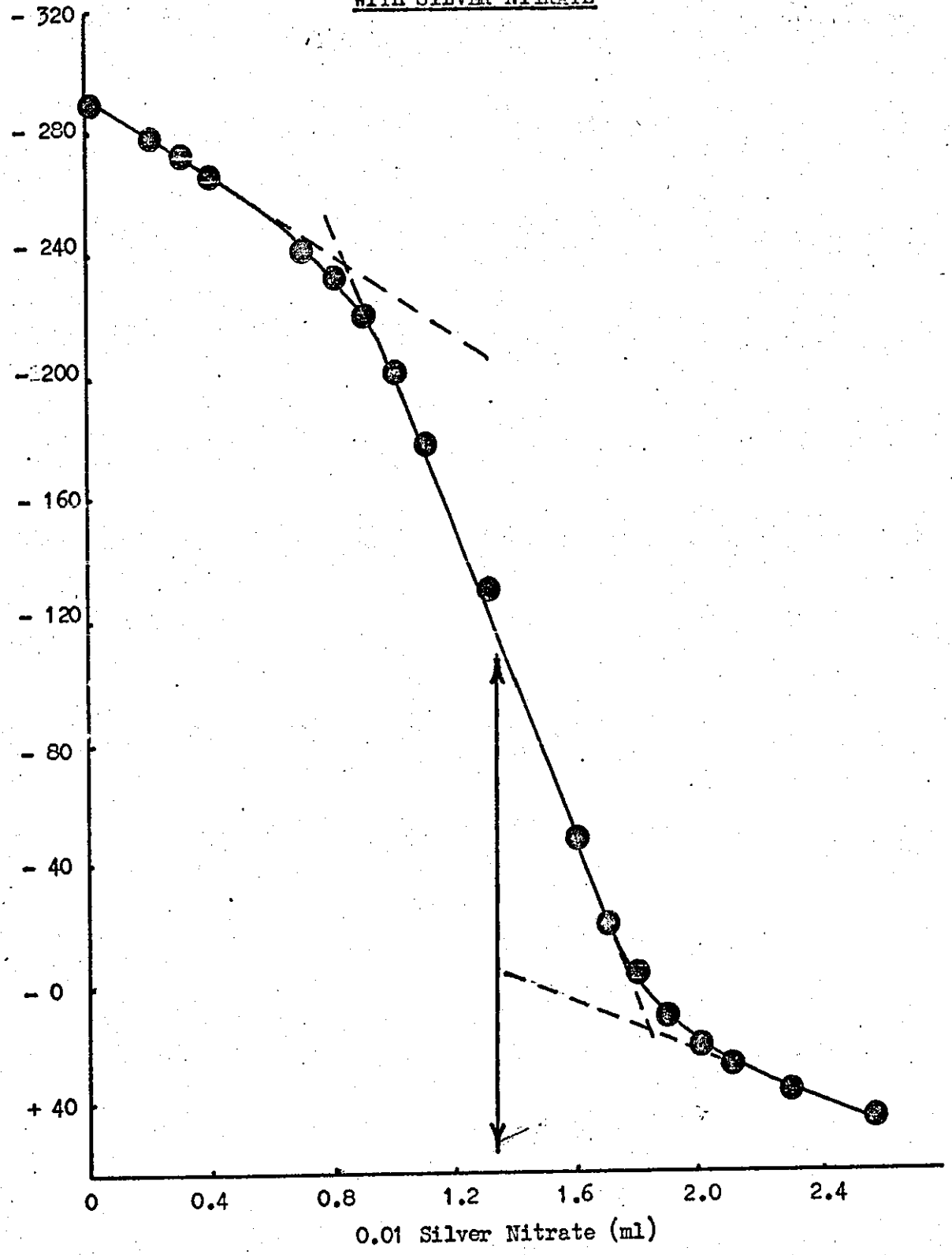
≡ 23.7 µg

This is the amount added to the sample purge flask.

TABLE A.1 - TYPICAL FIGURES OBTAINED FOR POTENTIOMETRIC TITRATION (USING n-PROPANE THIOL)

ml 0.01 N $AgNO_3$	EMF	ml 0.01 N $AgNO_3$	EMF
0	288	1.6	48
0.1	284	1.7	20
0.2	279	1.8	5
0.3	272	1.9	+ 8
0.4	266	2.0	18
0.5	259	21.	22
0.6	252	2.2	28
0.7	242	2.3	32
0.8	232	2.4	38
0.9	220	2.5	40
1.0	202	2.6	42
1.1	178	2.7	48
1.2	150	2.8	52
1.3	132	2.9	56
1.4	112	3.0	60

FIG. A.1 - POTENTIOMETRIC TITRATION OF PROPANETHIOL WITH SILVER NITRATE



APPENDIX B

THE STANDARDISATION OF DIMETHYL SULPHIDE SOLUTIONS

Reagents

Aq potassium bromate (approx. 0.02 N) - Potassium bromate (0.556 g) and potassium bromide (2 g), diluted to standard volume (1 litre) with water.

Sod. arsenite (0.01 N) solution - Arsenic (III) oxide (0.495 g) dissolved in 2 N sodium hydroxide (3 ml). 3 N hydrochloric acid (2 ml) is then added and the mixture cooled and diluted to standard volume (1 litre) with water. The pH of this solution must be 6.5 - 7.0 and is adjusted if necessary.

Hydrochloric acid - (36% w/w)

Aq methyl orange (0.025% w/v)

Methanol

Apparatus

Titration vessel - a glass vial (2 dram supplied by Johnsen & Jorgensen) fitted with a polythene cap.

Magnetic stirrer

'Agla' micrometer syringe - fitted with a stainless steel needle and glass capillary tip bent at right angles to pass through a hole in the polythene cap of the titration vessel. The syringe is clamped horizontally during the operation.

Procedure

Standardisation of aq potassium bromate

The following reagents are added to the titration vessel in the following order.

Aq standard arsenite (200 µl)

Water (0.8 ml)

Conc. hydrochloric acid (0.2 ml)

Methyl orange (1 drop)

Methanol (2 drops)

The titration assembly is warmed to 50°C in a water bath and the contents titrated with the aq potassium bromate solution until the indicator changes from pink to colourless.

A blank titration is determined by using water instead of the arsenite solution.

Standardisation of the dimethyl sulphide solution (back titration method)

Dimethyl sulphide (ca. 100 mg) is weighed into a tared conical flask (100 ml) containing methanol (35 ml). This solution is then diluted to standard volume (1 litre) and the dimethyl sulphide content determined as follows

The following reagents are added to the titration vessel in the order given viz

Water (0.5 ml)

Conc. hydrochloric acid (0.2 ml)

Aq standard potassium bromate so that an excess of 10 - 20 µl is present (1 µl of 0.02 N bromate ≡ 0.1603 µg for the oxidation of the sulphide to the sulphone).

Aq dimethyl sulphide (500 µl).

The above mixture is stirred for 1 minute at 50°C. Aq standard (150 µl) is added followed by methyl orange (1 drop) and methanol (2 drops). Titrate with aq standard bromate until indicator changes from pink to colourless.

Results (typical calculation)

Standardisation of aq bromate

Blank titre equals 5 µl of aq bromate (0.01 N). Titration of the standard arsenite required 106 µl for aq bromate (0.01 N).

∴ 200 µl AsO_3^{3-} (0.01 N) required 101 µl bromate

∴ normality of bromate = $\frac{200}{100} \times 0.01 = 0.0198$ N

Standardisation of dimethyl sulphide

Aq standard bromate added to titration vessel 220 µl Back titration of the added arsenite gave a bromate titre of 70.5 ul.

Amount (µl) of 0.02 N AsO_3^{3-} in excess of bromate

$= \frac{150 \times 0.01}{0.02} - (70.5 - 5) \times \frac{0.0198}{0.0200}$

$= 75 - 64.845$

$= 10.155$ µl of 0.02 N AsO_3^{3-}

$\therefore$ amount of aq standard bromate (0.02 N) used to oxidase dimethyl sulphide

$= (220 \times \frac{0.0198}{0.0200}) - 10.155 \times 0.1603$

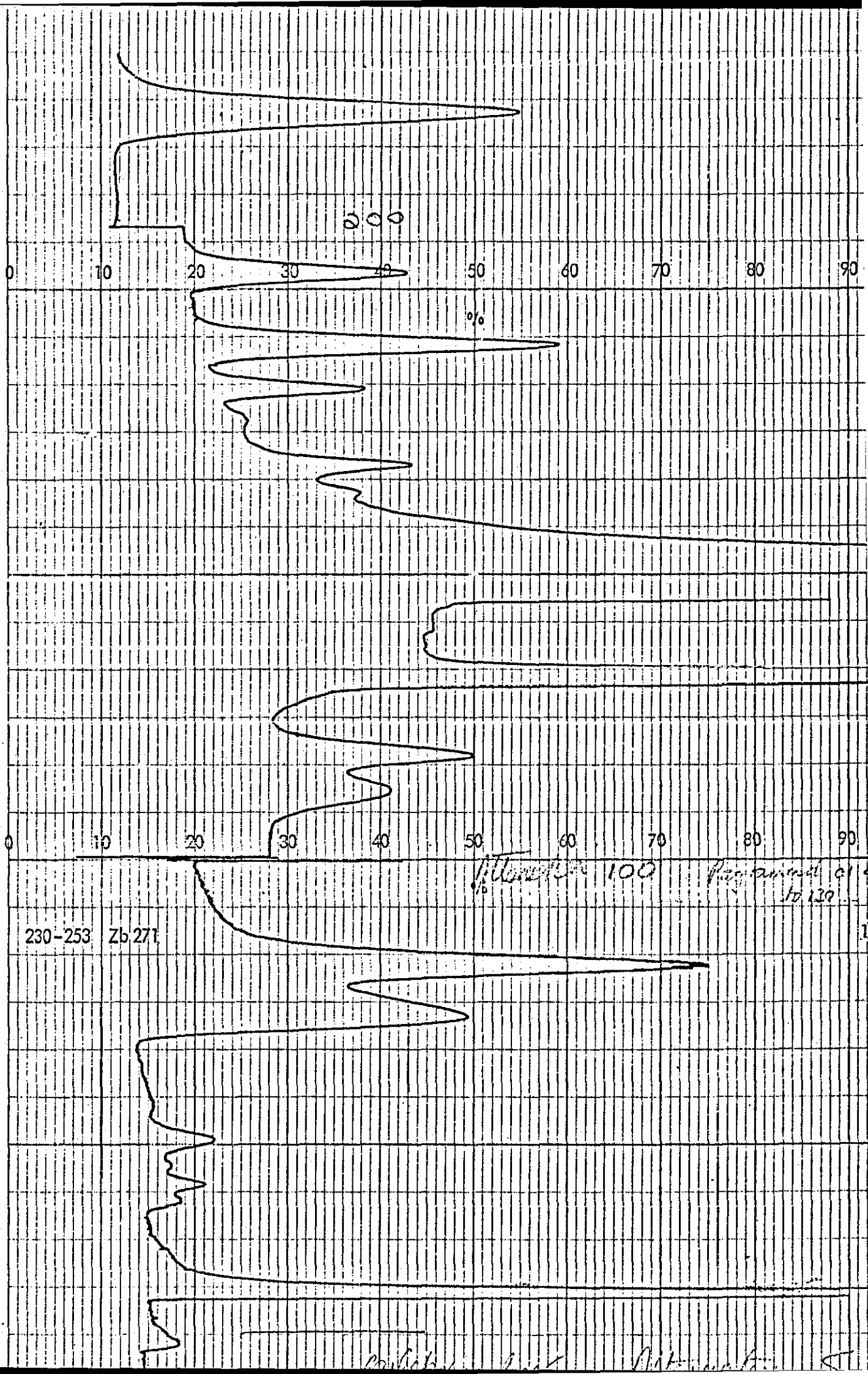
$=$ 33.3 µg DIMETHYL SULPHIDE (AS SULPHUR) PER 500 µl

APPENDIX C

GAS CHROMATOGRAMS ILLUSTRATING REMOVAL OF ACETALDEHYDE BY ADDITION OF HYDROXYLAMINE HYDROCHLORIDE

CHROMATOGRAM 1 - before addition of NH_2OH

CHROMATOGRAM 2 - after addition of NH_2OH


0
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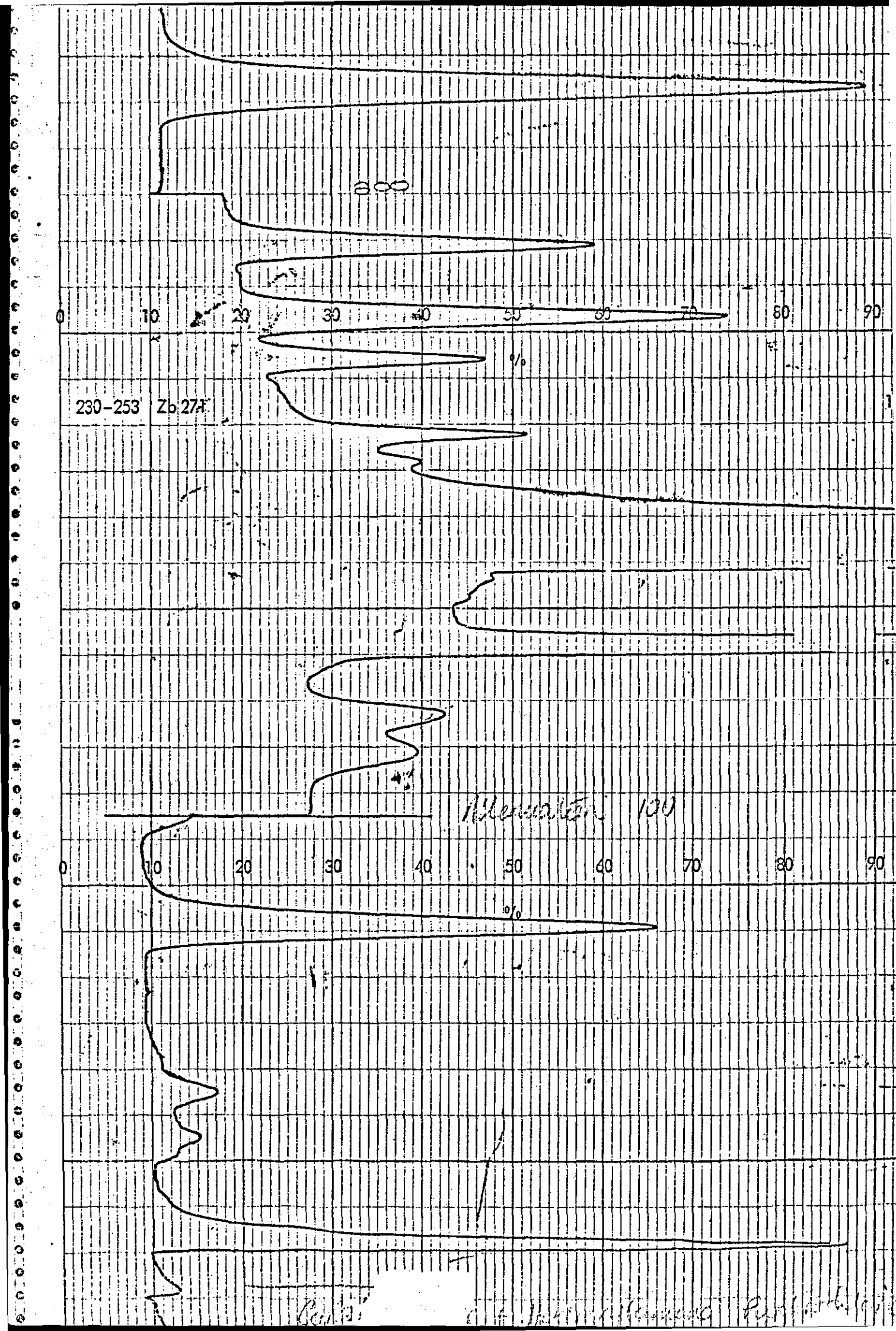
20
30
40
50
60
70
80
90
%
230-253 Zb 271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230–253 Zb 27 A
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