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# GAS–LIQUID CHROMATOGRAPHY OF THIOLS USING FLAME-PHOTOMETRIC DETECTION AND A DEACTIVATED TRANSFER LINE

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## SUMMARY

2-Mercaptobenzothiazole and benzotriazole have been assessed as reagents for the deactivation of the transfer line between a glass SCOT-capillary column and a flame-photometric detector operated in the sulphur mode. 2-Mercaptobenzothiazole was only moderately effective but coating with benzotriazole gave excellent results and proved suitable for both isothermal and temperature programmed analysis. The minimum detectable level achieved for butanethiol was $1.0 \cdot 10^{-10}$ g when a benzotriazole-treated line was used.

## INTRODUCTION

The major problem encountered in the gas chromatography of trace amounts of sulphur compounds is the loss which occurs in the analytical system. This loss, which is particularly acute in the case of thiols is caused by adsorption onto, or reaction with, components of the column[1–7]. A similar loss occurs in the transfer line to the detector[1,8–10].

Losses in the column may be reduced by careful choice of column material[1,3,6,7,11], support[1,3,4], and stationary phase[1,3] and then minimised by conditioning the system with repeated injections of the sample[4], or other suitable sulphur compounds[3,4,6]. In a few cases losses can also be diminished by use of carrier gas continuously doped with hydrogen sulphide or other sulphur compound[3].

Loss in the transfer line may be reduced by keeping this as short as possible or eliminated by fitting the column outlet into the base of the detector[1,11,12]. This is not always feasible, however, and the United Analysts flame-photometric detector (FPD) incorporates a considerable length of metal capillary between the column outlet and the detector base. In some other applications, the minimum length of the transfer line may also be appreciable. For example, if the column effluent is split between two detectors, it is exposed to a considerable area of active sites within pipework and fittings where losses may occur. In addition, in studies on food volatiles a transfer line to an odour port in the wall of the chromatograph oven, where the aroma of the eluted compounds may be assessed, represents a further example of

a moderately long line from which it is necessary to eliminate losses of sulphur compounds.

Since acceptable columns, in particular capillary columns employing polar phases, can fairly readily be prepared, we have used organic compounds which function as corrosion inhibitors to deactivate stainless-steel transfer lines. Whilst this study was in progress, Pearson[11] and Pearson and Hines[13] reported that the treatment of stainless steel with the water-soluble silanizing agent, Siliclad, markedly reduces loss of thiols, although he gave no figures to indicate the efficiency and permanency of the treatment.

## EXPERIMENTAL

### *Reagents*

Butanethiol and 2-mercaptobenzothiazole (2-MBT) were obtained from Aldrich (Gillingham, Great Britain), benzotriazole (BTA) from ABM (Stockport, Great Britain), hexamethyldisilazane (HMDS), Carbowax 20M and Atpet 80 from Phase Separations (Queensferry, Great Britain), Silanox 101 (particle size 6–10 $\mu$m) from Cabot (Boston, Mass., U.S.A.) and other reagents from BDH (Poole, Great Britain).

### *Chromatographic instrumentation*

The instrument used for gas chromatography was a hybrid composed of the following. Analyser oven and flame-ionization detector (FID) amplifier, Pye series 104 (Pye Unicam, Cambridge, Great Britain); oven temperature and temperature programme modules (Beckman, Fullerton, Calif., U.S.A.), modified in the workshop of the Electrical and Electronic Engineering Department, University of Leeds; FID, made in the workshop of the Procter Department and heated by conduction from the analyser oven; FPD, United Analysts (East Boldon, Great Britain) heated by conduction from the analyser oven and used with hydrogen and oxygen flow-rates of 100 ml/min and 6 ml/min, respectively.

### *Preparation of SCOT column*

Coiled glass capillary tubing (50 m × 0.5 mm I.D.) was prepared, using an apparatus of the Desty type[14] arranged for drawing and coiling capillary columns, from a length of Pyrex glass tubing (7 mm O.D. × 4 mm I.D.) which had previously been cleaned using detergent (Decon 75) solution. After annealing at 450° for 3 h and cooling, the capillary was connected to a glass pressure reservoir and dichloromethane (5 ml) passed through it by application of nitrogen pressure. Just before the last of the solvent had left the reservoir, a suspension of Silanox 101 (0.4 g) in dichloromethane (10 ml) containing Carbowax 20M (0.5 g) and Atpet (0.02 g) was added to the reservoir and allowed to flow slowly (*ca.* 10 cm/min) through the column by judicious adjustment of the nitrogen pressure. It was necessary to subject the suspension to ultrasonic vibration for 10 min to ensure adequate dispersion of the particles; it was also essential to ensure that no air bubbles were trapped in the coating suspension and that the movement of the suspension through the coiled tubing did not cease, otherwise blockage would almost certainly occur[15]. After the suspension had left the column, the latter was dried using a stream of nitrogen and conditioned at 180° until its performance was satisfactory.

*Chromatography*

The column was attached by means of heat-shrinkable PTFE tubing to a glass injection block as described by Cronin[16] and was used with nitrogen as carrier gas at a flow-rate of 4 ml/min. Chromatography was carried out isothermally at 93°, or with temperature programming from 60 to 180° at 2°/min. An injection temperature of 175° was used. The effluent was usually split between the FID and FPD in the ratio 1:1 by means of a stainless-steel tee coupling and stainless-steel capillary tubes (1.6 mm O.D. × 0.3 mm I.D.), although this communication refers only to the response observed on the FPD. On occasion, however, the total column effluent was diverted to the FPD by substituting a solid plug for the FID transfer line at the tee. The FPD line also possessed one section of greater diameter; the precise configuration of the links to the detector will be described later, together with the characteristics of the United Analysts detector.

*Deactivation of the transfer line*

*With hydrogen sulphide.* The line was removed from the analyser oven and a stream of hydrogen sulphide (generated from ferrous sulphide and hydrochloric acid) passed through it for 7 h. After heating at 150° in a stream of nitrogen the line was reconnected.

*With azoles.* The line and effluent splitter was removed from the analyser oven and connected to a reservoir. A solution (1% in acetone, 25 ml) of 2-MBT or BTA was allowed to flow under gravity so that the last portion of the solution remained in the line. After 1 h the liquid was replaced using fresh solution (25 ml) and again left to stand for 1 h. Replacement of the liquid was carried out three times more before the tube was rinsed with acetone (10 ml) and water (30 ml), conditioned by heating to 150° in a slow stream of nitrogen for 5 h and reconnected. The splitter was similarly treated by filling with the azole solution, which was changed hourly, and allowing to stand for 5 h before washing and conditioning as for the tube.

## RESULTS AND DISCUSSION

When small quantities of butanethiol (4.0 ng) were chromatographed using an untreated transfer line, none of the thiol reached the detector. Initially, attempts were made to deactivate the transfer line by passing octanethiol vapour through it and, although some improvement was noted, it was slight, short-lived and inconsistent. Thus, an attempt was made to eliminate active adsorption sites by treatment with the more reactive hydrogen sulphide. Continuous doping of column and transfer line to eliminate losses has been used by Perry and Carter[3]. A similar technique is not suited to the needs of this laboratory, where gas chromatographic effluents from complex mixtures of food volatiles are assessed sensorially, since, clearly, this cannot be carried out in the presence of hydrogen sulphide. The disturbance of the signal from a doped background, which is known to occur when compounds which do not contain sulphur are eluted[17,18], would also cause serious problems during the chromatography of aroma concentrates containing many such compounds. Accordingly, the transfer line was treated with hydrogen sulphide for a period of 7 h and reconnected in the apparatus. Fifteen aliquots (0.5 $\mu$l; 4.0 ng) of a freshly prepared solution of butanethiol (0.001%, v/v, in toluene) were chromatographed on a Car-

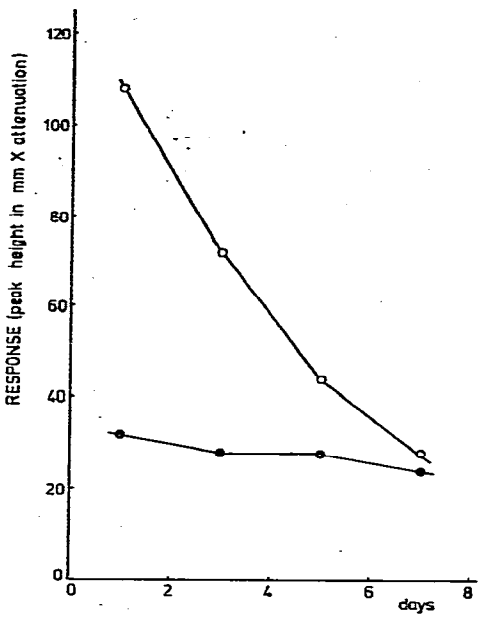


Fig. 1. Response of FPD to 4.0-ng injections of butanethiol after treatment of transfer line with hydrogen sulphide. ●, First treatment; ○, second treatment.

bowax 20M SCOT-capillary column. (The butanethiol sample available contained 5% dibutyl disulphide; thus 0.5 $\mu$l of a 0.001% (v/v) solution contained 4.0 ng, not 4.2 ng as would be obtained from pure butanethiol).

The first five injections were regarded as conditioning the column, the injection block, etc. The detector response from the next ten were noted, extreme values were rejected and the average of the eight remaining was calculated. The experiment was repeated on alternate days during 1 week using the same solution and a decrease of response with time observed (Fig. 1). Treatment of the transfer line with hydrogen sulphide was repeated and the detector response measured using a freshly prepared solution of butanethiol. The results (Fig. 1) showed an improvement over those from the initial hydrogen sulphide treatment but the response was still low and decreased with time. A part of the decrease was due to loss of thiol when the dilute solution was stored (see below) but, since hydrogen sulphide treatment was only partially effective, the experiment was not repeated using a fresh solution on each occasion.

Since treatment with hydrogen sulphide had proved only partially effective, deactivation using organic compounds which inhibit corrosion of metals was considered. The most useful corrosion inhibitors consist of a hydrocarbon residue attached to a polar or ionizable group and usually contain sulphur, nitrogen or oxygen atoms. Benzothiazole, BTA, 2-MBT, mercaptobenzimidazole and mercaptobenzoxazole are particularly effective inhibitors of corrosion in copper, copper alloys, iron and steel[19–22]. These compounds exhibit their protective effect through a layer adsorbed onto the metal surface[21–24], and, at least in the case of BTA and copper, chemisorption is involved[21,23,24]. No studies of the corrosion inhibition of stainless steel by these particular compounds have been reported although inhibition by

amines[25,26], quinoline[25,27], dibenzylsulphoxide[25,28], phenylthiourea[25] and a number of proprietary preparations[29] is known. However, since stainless steel clearly contains active adsorption sites, it appeared reasonable to anticipate that it might form stable complexes with high-boiling inhibitors of the azole-type with a consequent reduction in the number of sites available to accept thiols.

It is considered that reaction of azole-type corrosion inhibitors occurs, at least in the case of copper, through the nitrogen atom[30]. However, the compound, 2-MBT, first chosen also contained a thiol group in order to increase the possibility of its reaction with the stainless-steel surface.

After coating the transfer line and the effluent splitter with 2-MBT, fifteen aliquots (0.5 $\mu$l; 4.0 ng) of a freshly prepared butanethiol solution (0.001%, v/v, in toluene) were chromatographed and the average response calculated as above. As indicated in Fig. 2, the FPD response was considerably greater than when the line was treated with hydrogen sulphide, but it decreased by approximately 25% during the course of one week. This decrease was largely due to loss of the thiol from the dilute solution during storage since the response was largely restored when a freshly prepared solution was chromatographed at the beginning of the second 7-day period*.

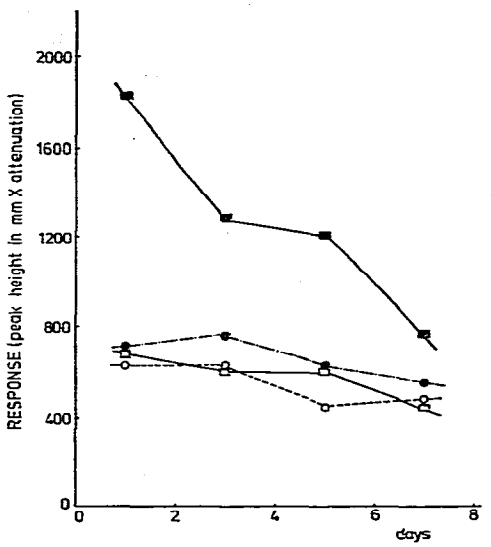


Fig. 2. Response of FPD to 4.0-ng injections of butanethiol after treatment of transfer line with 2-MBT. ●–·–·–●, First week of first coating; ○—○, second week of first coating; □—□, third week of first coating; ■—■, first week of second coating.

During the second 7-day period and in all subsequent experiments, either a freshly prepared solution was used each day or a correction made for the loss of butanethiol which occurred upon storage (solutions were stored for not more than one week, by which time the response had fallen by about 17%, corresponding to a butanethiol loss of approximately 8%). Loss of thiols has been encountered in gas mixtures by

* The 7-day periods were not quite consecutive but were separated by 2–3 days.

several authors[31–33] but not, apparently, in solution. The loss was mainly due to oxidation giving dibutyl disulphide, although this compound was not detected when chromatography was carried out at 93° because it was present at less than its minimum detectable level (MDL) at that temperature[34]. However, when a solution which had been stored for one week was examined under other conditions, it was found that dibutyl disulphide equivalent to approximately 6% of the initial thiol concentration had formed during storage[34].

A real loss of response was, however, observed during the second and third 7-day periods (Fig. 2), suggesting that some of the 2-mercaptobenzothiazole was bleeding from the transfer line and reducing its efficiency. Confirmation that bleeding was occurring was given by the presence of a small negative peak for the solvent (toluene) during the first week. The negative peak was, on average, twice as large during the second 7-day period and larger again by a factor of *ca.* 2.5 during the third. Small negative peaks for the solvent were also noted when the transfer line was treated with hydrogen sulphide. The appearance of a negative peak for a hydrocarbon on a steady base line when using a FPD is diagnostic of a bleed containing sulphur. It results from the partial quenching, by the hydrocarbon, of the steady emission from the sulphur[7,17,18,32].

A further consequence of the presence of a bleed containing sulphur is that the response of the FPD to eluted sulphur compounds is enhanced; in fact, deliberate introduction of a steady sulphur bleed can be used as a means of increasing the sensitivity of the detector[17,18]. This is because the signal from the FPD is proportional, not to the amount of sulphur passing through it, but to the *n*th power of that amount (when *n* is usually 1.5–2.0 and theoretically should be 2). Thus, not only is the response enhanced, but small amounts of sulphur are overemphasised relative to larger ones and so the values recorded for butanethiol, particularly at the end of the second and subsequent 7-day periods, are probably somewhat high*; the same reason may account for the increase in response between the end of the second week and the beginning of the third.

The transfer line was again treated with 2-MBT and the behaviour of a freshly prepared butanethiol solution examined as above. The initial response (Fig. 2) was considerably higher than after the initial coating but fell off very sharply during 7 days to a value little higher than that obtained after the first coating, thus indicating the second coating to be less stable than the first. The negative solvent peak was of a size similar to that observed during the third 7-day period following the initial coating; thus the FPD response is likely to be erroneously high by a similar amount.

The bleed indirectly observed at 93° indicated that the coating was unlikely to be suitable for temperature programmed studies and this was confirmed by injecting pentane (1 $\mu$l) onto the column and raising the oven temperature from 65° to 150° at 2°/min when a rapidly rising base line was observed. After 3 repetitions,

* It has been stated[3] that when a background due to sulphur is present, the response to samples is then approximately proportional to their concentration, rather than to the square of their concentration. This is only true when the sample response in the absence of background is less than 10–20% of the background response[17]; when the background response is low, *e.g.* of the same order as that given by the smaller samples entering the detector, the effect is to enhance (on the basis of response proportional to the square of the concentration) low values relative to higher ones.

the FPD response to 4.0-ng injections of butanethiol was only 4–5% of that observed prior to carrying out temperature programming.

With weekly recoating, 2-MBT deactivation of the transfer line would be useful for isothermal studies at or below 93°, although not without disadvantages. However, it is clearly unsuited to the temperature-programmed studies necessary in flavour analysis. Attention was thus turned to BTA in the hope that this would be more resistant to removal due to bleeding and, since it is sulphur-free, any slight bleeding which might occur would not affect the response given by the FPD.

The transfer line and associated couplings were well washed using acetone, dried and internally coated with BTA to give line $l_{BTA}$. The efficiency of deactivation of $l_{BTA}$ was assessed as described for 2-MBT and found to be considerably higher (Fig. 3); response to the standard injection fell slightly throughout the first 7-day period but remained approximately constant during the second 7-day period.

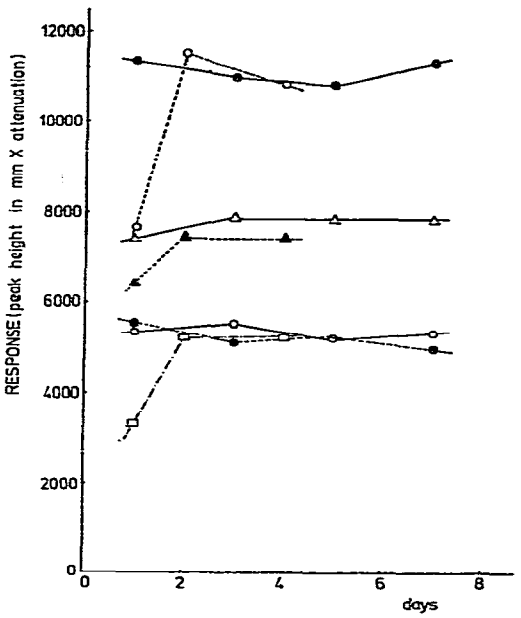


Fig. 3. Response of FPD to 4.0-ng injections of butanethiol after treatment of transfer line with BTA. ●—●, Line $l_{BTA}$, first week of first coating; ○—○, line $l_{BTA}$, second week of first coating; □–·–·–□, line $l_{BTA}$, first week after carrying out temperature programme sequence; △—△, line $l_{BTA}$, first week of second coating; ▲—▲, line $l_{BTA}$, first week after carrying out temperature programme sequence subsequent to second coating; ●—●, line $2_{BTA}$, first week after coating; ○—○, line $2_{BTA}$, first week after carrying out temperature programme sequence.

Suitability of the BTA coating in conjunction with temperature programming was assessed by carrying out four temperature programmed analyses (65–150° at 2°/min) using pentane (1 $\mu$l). The response to the standard butanethiol injection, determined immediately after programming, had fallen markedly (by 36%) but by the second day the coating had recovered almost all of its efficiency (Fig. 3). The loss of efficiency on temperature programming and its recovery following repeated injec-

tion of butanethiol (*i.e.* by day 2) may, in fact, be largely due to the uncovering, and recovering, of active sites within the column rather than the transfer line. After recoating with BTA the situation was even more satisfactory, a stable coating being obtained which afforded a higher response to the butanethiol solution (Fig. 3) and one which improved somewhat between days 1 and 3 and then remained constant.

Immediately after again performing the temperature programming sequence the response to butanethiol had fallen by 12%, but virtually all of this had been regained by the second day after ending the sequence (Fig. 3). At this time the MDL for butanethiol was $2.0 \cdot 10^{-10}$ g ($1.2 \cdot 10^{-11}$ g sulphur/sec) as compared with a value of $8.0 \cdot 10^{-9}$ g ($2.4 \cdot 10^{-10}$ g sulphur/sec) using the uncoated line.

The BTA-coated transfer line $l_{BTA}$ was used in conjunction with temperature-programmed gas chromatography of food volatiles for 6 months and was recoated once during that time. The performance of the line was still satisfactory at the end of this period, the MDL of butanethiol then still being $2.0 \cdot 10^{-10}$ g. This value relates to the system in which the column effluent was equally divided between FPD and FID. When the total effluent was directed to the FPD, the MDL became $1.0 \cdot 10^{-10}$ g ($5.9 \cdot 10^{-12}$ g sulphur/sec). The precise halving of the MDL when all the column effluent passed through the transfer line indicates that any losses which occur in the injection block and column must be small.

The BTA-deactivated line, $l_{BTA}$, had previously been coated with 2-MBT and cleaned with acetone before treatment with BTA. If any chemisorption of 2-MBT to the line had occurred, this would not have been reversed during the cleaning procedure and the line would therefore have been deactivated partly by 2-MBT and partly by BTA.

In order to obtain a line known to be entirely deactivated by BTA, a new transfer line and associated couplings were thoroughly cleaned using, successively, dichloromethane, concentrated nitric acid, water and acetone, dried and coated to give line $2_{BTA}$. The efficiency of deactivation of $2_{BTA}$ was assessed using butanethiol as above when the response obtained (Fig. 3) was 52% higher than that for line $l_{BTA}$. After four temperature programmed runs had been performed, the efficiency had fallen somewhat, but had been fully recovered by the second day.

Although the response to a 4.0 ng injection of butanethiol was considerably enhanced using the line $2_{BTA}$, the MDL of the thiol was the same ($2.0 \cdot 10^{-9}$ g when the effluent was divided between the two detectors and $1.0 \cdot 10^{-9}$ g when the total effluent was directed to the FPD) as when line $l_{BTA}$ was used. The reason for the failure of an increased response to be matched by an improvement in the MDL is not obvious. However, it does indicate the probability that loss in the transfer line is no longer the limiting factor and that improvements would have to be made elsewhere, probably within the detector itself, to obtain greater sensitivity. It may be that the presence of 2-MBT thiol groups in the line $l_{BTA}$ may have caused a very slight increase in the butanethiol peak width which did not occur when line $2_{BTA}$ was used, although any such difference was too small to be detected by measurement. In contrast, the small peak obtained at the MDL is, by its very nature, relatively broad and any effect due to the presence of 2-MBT in the transfer line likely to be negligible.

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REFERENCES

1 R. K. Stevens, J. D. Mulik, A. E. O'Keeffe and K. J. Krost, *Anal. Chem.*, 43 (1971) 827.
2 H. E. Hansen, J. Strating and W. M. Westra, *J. Inst. Brew.*, 77 (1971) 154.
3 S. G. Perry and F. W. G. Carter, in R. Stock (Editor), *Gas Chromatography*, Institute of Petroleum, London, 1971, p. 381.
4 W. E. Rupprecht and T. R. Phillips, *Anal. Chim. Acta*, 47 (1969) 439.
5 P. Ronkainen, J. Denslow and O. Leppänen, *J. Chromatogr. Sci.*, 11 (1973) 384.
6 W. Bertsch, F. Hsu and A. Zlatkis, *Anal. Chem.*, 48 (1976) 928.
7 L. Blomberg, *J. Chromatogr.*, 125 (1976) 389.
8 R. K. Stevens, A. E. O'Keeffe and G. C. Ortman, *Envir. Sci. Technol.*, 3 (1969) 652.
9 G. A. F. Harrison and C. M. Coyne, *J. Chromatogr.*, 41 (1969) 453.
10 C. J. Cowper, in R. Stock (Editor), *Gas Chromatography*, Institute of Petroleum, London, 1971, p. 397.
11 C. D. Pearson, *J. Chromatogr. Sci.*, 14 (1976) 154.
12 F. Bruner, P. Ciccioli and F. Di Nardo, *Anal. Chem.*, 47 (1975) 141.
13 C. D. Pearson and W. J. Hines, *Anal. Chem.*, 49 (1977) 123.
14 D. H. Desty, J. N. Haresnape and B. H. F. Whyman, *Anal. Chem.*, 32 (1960) 302.
15 M. H. Jee, *Ph.D. Thesis*, University of Leeds, Leeds, 1977.
16 D. A. Cronin, *J. Chromatogr.*, 97 (1974) 263.
17 A. R. L. Moss, *Scan*, 4 (1974) 5.
18 W. L. Crider and R. W. Slater, *Anal. Chem.*, 41 (1969) 531.
19 T. G. Neznamova and V. P. Barannik, *Dopovidi Akad. Nauk. Ukr. RSR*, (1966) 1176; *C.A.*, 66 (1967) 31349n; and (1966) 1451; *C.A.*, 66 (1967) 40064t.
20 B. Sathianandhan, K. Belakrishnan and H. Subramanyan, *Brit. Corros. J.*, 5 (1970) 270.
21 F. Mansfield, T. Smith and E. P. Parry, *Corrosion.*, 27 (1971) 289.
22 S. Thibault and J. Talbot, *C.R. Acad. Sci., Ser. C*, 278 (1974) 503.
23 J. B. Cotton and I. R. Scholes, *Brit. Corros. J.*, 2 (1967) 1.
24 G. W. Poling, *Corros. Sci.*, 10 (1970) 359.
25 V. Carassiti, G. Trabanelli and F. Zucchi, *Proc. 2nd Eur. Symp. Inhibitors Corros., Ferrara, 1965*; *Annali Univ. Ferrara, Sez. 5*, Supp. 4 (1966) 417.
26 J. Horváth, A. Rauscher, L. Hackl and F. Márta, *Proc. 3rd Eur. Symp. Inhibitors Corros., Ferrara, 1970*; *Annali Univ. Ferrara, Sez. 5*, Supp. 5 (1971) 851.
27 Z. Ahmad and J. C. Scully, *Proc. 3rd Eur. Symp. Inhibitors Corros., Ferrara, 1970*; *Annali Univ. Ferrara, Sez. 5*, Supp. 5 (1971) 195.
28 G. L. Zucchini, R. Zucchi and G. Trabanelli, *Proc. 3rd Eur. Symp. Inhibitors Corros., Ferrara, 1970*; *Annali Univ. Ferrara, Sez. 5*, Supp. 5 (1971) 577.
29 W. Machu, *Proc. 3rd Eur. Symp. Inhibitors Corros., Ferrara, 1970*; *Annali Univ. Ferrara, Sez. 5*, Supp. 5 (1971) 107; R. L. Tama Char, *Proc. 3rd Eur. Symp. Inhibitors Corros., Ferrara, 1970*; *Annali Univ. Ferrara, Sez. 5*, Supp. 5 (1971) 147.
30 S. Thibault and J. Talbot, *Met. Corros. Ind.*, 50 (1975) 5.
31 M. Feldstein, S. Balestrieri and D. A. Levaggi, *J. Air Poll. Contr. Ass.*, 15 (1965) 215.
32 K. A. Goode, *J. Inst. Petrol., London*, 56 (1970) 33.
33 B. H. Devonald, R. S. Serenius and A. D. McIntyre, *Pulp Pap. Mag. Can.*, 73 (1972) 50.
34 J. W. Gramshaw and A. Hussain, in preparation.