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Analytical Instrument Performance Criteria

Measurement of Biocides in Metalworking Fluids and in Workplace Air Using Capillary Electrophoresis

Kevin Ashley, Column Editor

Reported by Petra Fischer, Kai Hansen, and Dietmar Breuer

Water-mixed cooling lubricants, or metalworking fluids, provide excellent environments for microorganisms to grow. Numerous studies over the last few decades have shown that the conditions for the growth of microorganisms are quite good in cooling lubricants.[1,2] Diverse species of bacteria, yeasts, and fungi have been isolated in cooling lubricant emulsions. Aerobic bacteria can grow in the ventilated parts of a cooling lubricant circulation system (see Figure 1). Anaerobic microorganisms are also often found growing on inner surfaces and in undisturbed areas within machines.

Cooling lubricants contaminated with bacteria or fungi can cause numerous problems, beginning with the unpleasant smell of these emulsions (commonly known as "Monday odour" in metalworking industries in Germany), to production difficulties and health hazards resulting from skin contact or inhalation of the associated aerosols. Because it is not possible to designate an entirely harmless concentration of biological contamination, the microbial content in cooling lubricants should be as low as possible.

Biocides with germ-killing effects at low quantities are added to metalworking fluids to reduce the microbial presence.[3] If the biocides are added to the cooling lubricants in concentrations that are too high, these compounds themselves can also lead to health risks including skin inflammation and respiratory irritation.[4] Substances employed as biocides often utilize the germ-killing effects of nitrogen-oxygen and/or nitrogen-sulphur aromatic heterocycles; some example compounds are shown in Table I. Non-aromatics such as bismorpholinomethane (BMM) or hexahydrotriazine (HHT), which react to produce formaldehyde, are also used (see Table I). Most of the analytical methods for determining these substances use high-performance liquid

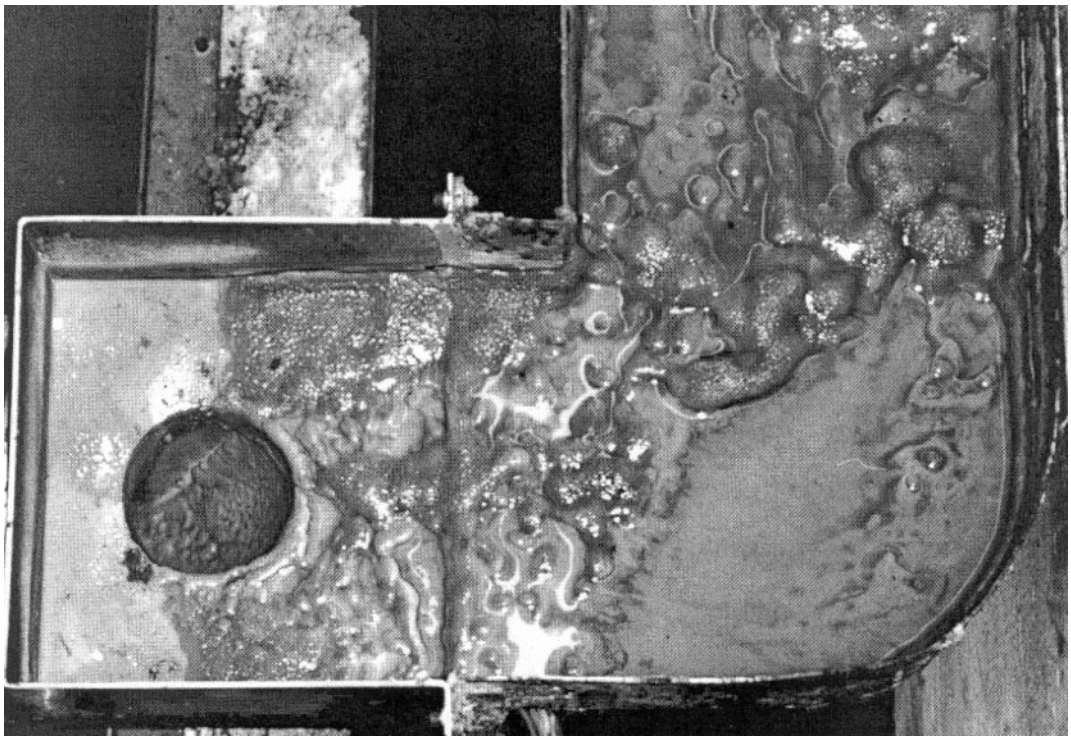
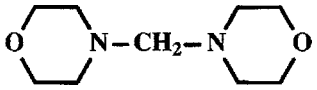
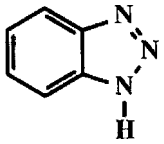
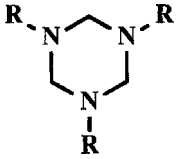
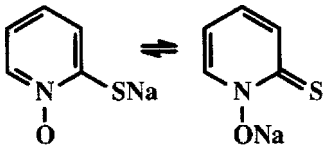
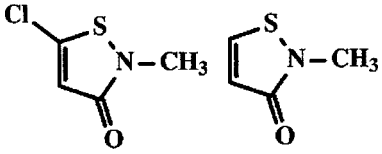
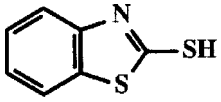
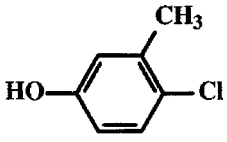


FIGURE 1
Biofilm in metalworking fluids.

TABLE I
Examples of biocides and corrosion inhibitors

Biocide/corrosion inhibitor	Limit value	Notations
 Bismorpholinomethane (BMM)	—	—
 Benzotriazole (BTA)	—	—
 Hexahydrotriazine (HHT)	—	—
 Sodium pyrithion	1 mg/m^3 [A] (DFG)[B]	Skin
 2-Chloro-2-methyl-2,3-dihydrothiazol-3-one; 2-Methyl-2,3-dihydrothiazol-3-one	0.05 mg/m^3 0.2 mg/m^3 [A] (DFG)	Skin
 2-Mercaptobenzothiazole	4 mg/m^3 [A] (DFG)	Suspected human carcinogen (DFG); Skin
 4-Chlor-3-methylphenol	—	—

[A] Sampling as inhalable aerosol.
[B] Deutsche Forschungsgemeinschaft: List of MAK (chemical exposure limits) and BAT (biological exposure limits) values.(4)

chromatography (HPLC) as the measurement technology, but HPLC often has the disadvantage of requiring complicated sample preparation procedures.

A large number of these biocide compounds can be successfully analyzed using capillary electrophoresis (CE) under the proper conditions. The advantages of CE lie mostly in the ease of sample preparation, low consumption of reagents, high separation performance, and very short periods of analysis. The devices currently available are very robust and simple to automate.

However, there are some disadvantages to CE, such as the use of a diode array detector (DAD) as a standard detector; to date, commercial CE instruments interfaced with mass spectral detectors have technical difficulties. Also, there are strict requirements on buffer composition used for CE. Additionally, because of the very small diameters of the capillaries used for separation, CE is often not suited for trace analysis.

Methods

CE has proven itself in particular for measuring biocides in cooling lubricants, in part because the advantage of simple sample preparation is especially highlighted.[5] In using HPLC methods to study cooling lubricants, numerous other compounds — and the mineral oil portions in particular — often stand in the way of routine sample preparation. Because of complicated sample preparation requirements, HPLC is not regarded as a satisfactory analytical method for measuring the numerous compounds that may be present in cooling lubricants. Hence, CE has been investigated as an alternative analytical technique for measuring biocides in metalworking fluids.

Biocides and Corrosion Protection Agents

A number of biocides and corrosion protection agents were tested to determine whether they could be separated and analyzed using CE. It was found that, under the conditions described in Table II, the separation of the following substances is possible: bithionol, sodium pyrithion, 1H-benzotriazole, 5-methyl 1H-benzotriazole, 5,6-dimethyl-1H-benzotriazole, mercapto benzothiazole, p-chlorine-m-cresol, and p-tert-butyl benzoic acid. Initial results indicated, however, that bithionol and sodium pyrithion could only be measured qualitatively. Results for bithionol illustrated a broad peak and could not be reproduced, and sodium pyrithion showed a decomposition reaction.

The otherwise quite insensitive signal for sodium pyrithion comes almost at the same retention time as the signal from 5-methyl-1H-benzotriazole, whereas the decomposition product is excellent for qualitative determination, thanks to its much greater ultraviolet (UV) sensitivity. Recorded over several days, the electropherograms showed both signals from a sodium pyrithion standard. It can be clearly seen that a second large peak appears over time, while the signal intensity for non-decomposed sodium pyrithion falls to a negligible degree (see Figure 2). A few days later, a considerably enlarged peak for the decomposition product is apparent alongside a slightly reduced signal for the original product.

The electropherogram in Figure 3 shows the separation of the seven substances: 1H-benzotriazole (BTA), 5-methyl-1H-benzotriazole (MBA), 5,6-dimethyl-1H-benzotriazole (DMBA), mercapto benzothiazole (MBT), p-chloro-m-cresol (CMP), p-tert-butyl benzoic acid (ptBBA), and bithionol (BiTL). It can be seen that the peaks for these seven compounds are well resolved. However, it was found that benzoic acid and MBT were not separable under the conditions of the analysis. Calibrations were made for five biocides and corrosion protection agents: BTA, MBA, DMBA, MBT, and ptBBA. The coefficients of variation for the CE analysis procedure ranged between 0.8 and 1.5 percent, and the correlation coefficients were all better than 0.999. The limits of quantification determined from the calibration data for all biocides were under 1 μg/milliliter (ml). The data gathered from standard solutions showed the CE method to be effective for determining the contents of these compounds in aqueous solutions.

TABLE II
Capillary electrophoretic conditions for the separation of biocides and corrosion protection agents

Electrophoretic system	Three-dimensional commercial CE system with diode array detection (DAD) and interfaced to personal computer
Capillary	Extended light path capillary, i.d. 50 μm; L_{abs} 48.5 cm; L_{eff} 40 cm; bubble factor 3–5
Buffer solution	CAPS (3-[Cyclohexylamino]-1-propansulfonic acid), conc. = 0.025 M; CH_3CN = 15% (v/v); pH = 11.75
Temperature	25°C
Rinsing	4 minutes with buffer solution
Voltage	23.5 kV
Electrical current strength	20 μA
Injection	Pressure injection, 500 hPa × s (50 hPa, 10 s)
Replenish	After each 6th analysis
DAD measuring wavelength	236 nm, 254 nm, 277 nm

Studies on Cooling Lubricants

Overall, 36 mineral oil–containing cooling lubricant concentrates from different manufacturers produced during the years 2000 to 2002 were studied; we targeted those cooling lubricants whose material safety data sheets suggested the presence of biocides. In preparing the samples, 1 gram (g) of each of the cooling lubricants was placed in a 100-ml volumetric flask and diluted with approximately 50 ml of water. The resulting emulsions were treated for 15 minutes in an ultrasonic bath. The sonicated samples were then filled with water to the calibration mark and mixed. Aliquots of the treated samples then only had to be filtered and transferred to the auto-sampler vial and could be analyzed

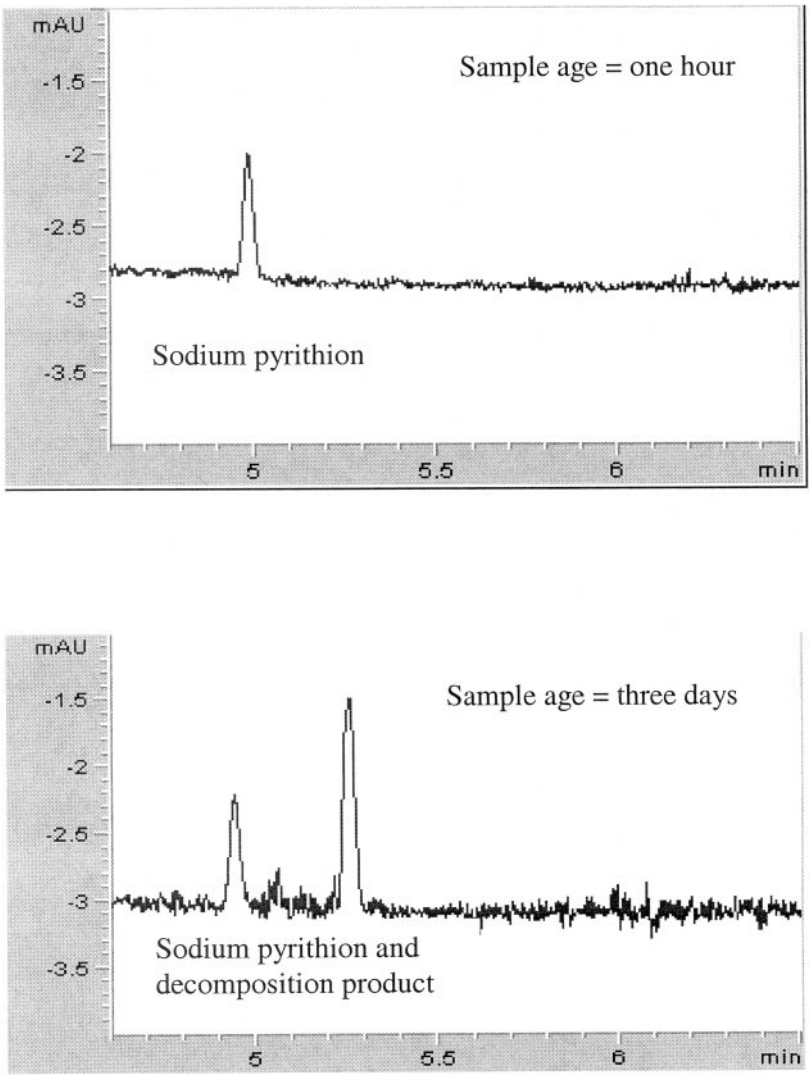


FIGURE 2
Decomposition of sodium pyrithion.

immediately. As already mentioned, this is a very simple preparation procedure, because separating the mineral oil portion from the rest of the cooling lubricants is not required.

In 26 of the samples, one or more of the biocides were present: benzotriazoles (MBA, 14 samples; BTA, 7 samples), mercapto benzothiazole (1 sample), sodium pyrithion (8 samples), and benzoic acid derivatives (6 samples). The concentrations of benzotriazoles were generally found to be in the range of 0.05 to 0.3 percent (w/w). In one case, however, an extremely high concentration of 9.2 percent was found.

The quantitative evaluation of these samples was done using the standard addition procedure in order to eliminate matrix effects as much as possible. In addition, the purity of the signal was inspected by comparing the spectra of the peaks with those of the standard solutions. An electropherogram of one sample containing MBT and sodium pyrithion showed that the decomposition product and an otherwise unidentifiable substance were both present. The benzotriazole concentration found here using the standard addition procedure was 0.03 percent. Under the given analytical conditions, biocide concentrations of approximately 5 to 10 mg/kg can be determined in the cooling lubricants. It is also possible to lower the dilution factor, but it is nearly impossible to quantitate below 1 mg/kg.

Air Sampling

The biocides p-tert-butyl benzoic acid, mercapto benzothiazole, and sodium pyrithion have regulatory limit values in Germany (see Table I). After determining the suitability of the CE method for use on aqueous solutions, the method was to be extended for analyzing air samples.

The first procedure attempted was to capture the biocides using water. To do this, two impingers were filled with 20 ml of water and set up in series. The biocides were captured by the water as air was passed through. However, the aqueous solutions tended to foam quite heavily, even at a low biocide concentration. Hence, it was decided to try an alternative sampling scheme.

Because all the substances of interest were present as solids at room temperature, it was felt that the samples could be taken successfully using a filter. With the exception of p-chlorine-m-Kresol (CMP), all the biocides were collected with a quartz-fiber filter in quantities satisfactory for identification. Although CMP could not be found on the filters, once an adsorption test tube was placed next in series, CMP recovery rates of up to 50 percent could be determined (depending on the packing materials). Activated charcoal, silica gel, and different organic adsorbents (such as XAD-2) were tested. The biocides sodium pyrithion and bithionol were also taken into consideration at first, but it was soon discovered that the results for these species could not be reproduced.

Ultimately, we were able to devise a method for determining five biocides collected on quartz-fiber filters. Sampling was done with samplers used in Germany for collection of inhalable aerosols, at an air sampling rate of 1.0 liters per minute.[6] Immediately after sample collection, the filters were removed from the sampler and placed in

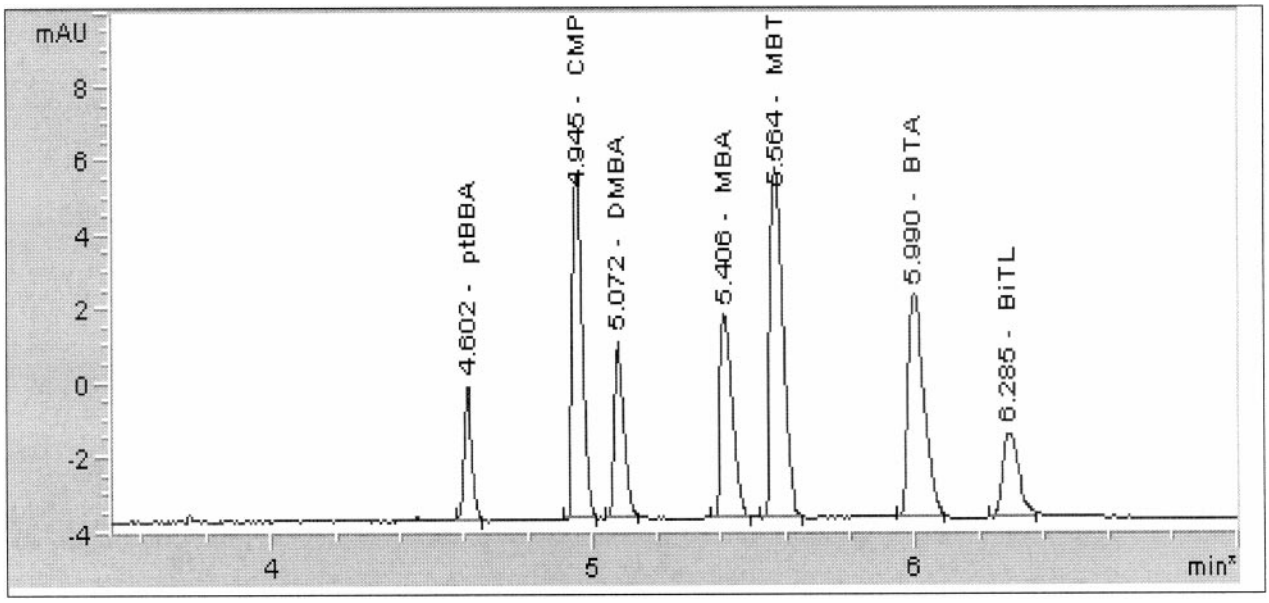


FIGURE 3
Electropherogram of biocides.

brown glass bottles filled with 10 ml of water; this precaution almost completely eliminated any loss from sample transport.

Validation of the method for each analyte was carried out to the extent possible. Analytical data were, in some instances, varied given the low observed recoveries for ptBBA and MBT at certain concentration levels (see Table III). However, recoveries for the three benzotriazoles were all near 100 percent. The reproducibility for most substances was better than five percent (see Table III), with one exception for low-level mercapto benzothiazole.

Outlook

The CE procedure for determining biocides in the air in work environments was presented to the German

TABLE III
Validation data

Substance	Working range[A] [mg/L]	Concentration [mg/m^3]	Recovery [%]	Coefficient of variation [%]	Limit of quantification [mg/m^3]
Benzotriazole	1–10	0.208	98	2.1	0.1
		2.08	98	1.7	
		4.17	103	1.4	
5-Methyl-1H-benzotriazole	1–10	0.208	103	1.6	0.1
		2.08	100	1.5	
		4.17	103	1.2	
5,6-Dimethyl-1H-benzotriazole	1–10	0.208	104	2.3	0.1
		2.08	99	1.3	
		4.17	101	1.7	
Mercapto-benzothiazole	1–10	0.208	27	16.0	(∼1.0)
		2.08	89	3.2	
		4.17	87	2.7	
p-tert-butyl benzoic acid	1–10	0.208	92	3.5	(∼0.1)
		2.08	83	2.2	
		4.17	63	4.5	

[A]Working range for the measuring solution; higher concentrations must be diluted.

committee responsible for publishing the recognized analytical methods for substances in workplace air. As part of the publication process, newly developed procedures are always tested by independent laboratories. Since the tests of the method by outside laboratories were successful, the CE method described herein was approved, and publication can be expected. The English translation is to be printed in the next edition of the series, "Analyses of Hazardous Substances in Air."[7]

To further our inquiry into measuring biocides in cooling lubricants, we are currently working on a method for determining isothiazolones (5-chlor-2-methyl-2,3-dihydroisothiazol-3-one-CMI; 2-methyl-2,3-dihydroisothiazol-3-one-MI, 5-octyl 2-methyl-2,3-dihydroisothiazol-3-one) using CE. Preliminary results appear to be quite promising.

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