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DEVELOPMENT OF A SIMULTANEOUS GAS CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF COPPER, NICKEL AND VANADIUM AT THE ULTRA-TRACE LEVEL

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SUMMARY

The development of a sensitive gas chromatographic method for simultaneously determining trace amounts of copper, nickel and vanadium in biological materials is described. The reagent is the fluorinated Schiff base 1,1,1,1′,1′,1′-hexafluoro-4,4′-(1-methylethane-1,2-diyldiimino)bis(pent-3-en-2-one), which is readily prepared in its isomerically pure form. Suitable column components are identified and the stability of chelates and optimum chromatographic conditions are discussed. Detection limits are about 5 pg for each of these elements. Some preliminary analytical results are also presented.

INTRODUCTION

It is almost three decades since gas chromatography (GC) was seen as a potential tool for the analysis of metals[1], yet the list of metals that can be successfully quantified at ultra-trace levels by GC remains comparatively short. In retrospect, the versatility of the β-diketones has proved useful for only a few metals[2–5] such as beryllium, chromium and aluminium. This list has been extended, by employing a modification of the β-diketone structure, to include the determination[6] of nickel with the monothio analogue of 1,1,1-trifluoropentane-2,4-dione and, with derivatives of β-diketones, to determine vanadium[7], copper and nickel[8] determined as chelates of the tetradentate β-ketoenamines. Other than these, only the structurally unrelated dialkyldithiocarbamates appeared promising enough to be applied to analyses of copper, nickel and zinc[9].

Requirements for the GC of neutral, volatile and thermally stable chelates restrict its wider applicability so that while the search for ideal complexing reagents continues, it is necessary to accept the limited value of existing ligands and optimize the chromatographic conditions for their use. Accordingly, this paper presents the results of an attempt to develop systematically a method for the simultaneous de-

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termination of copper, nickel and vanadium in biological matrices. The ligand 1,1,1,1′,1′,1′-hexafluoro-4,4′-(1-methylethane-1,2-diyldiimino)bis(pent-3-en-2-one) (H_2tfapd) is conveniently prepared by the condensation of 1,1,1-trifluoropentane-2,4-dione and 1,2-diaminopropane for this purpose. It reacts readily with trace amounts of copper and nickel but not vanadium. The detection limits for these chelates are about 5 pg.

EXPERIMENTAL

Syntheses

The ligand 1,1,1,1′,1′,1′-hexafluoro-4,4′-(methylethane-1,2-diyldiimino)bis-(pent-3-en-2-one) (H_2tfapd) and its microanalytically and isomerically pure metal chelates, M-tfapd where M = Cu(II), Ni(II), Pd(II) and VO(IV), were synthesized according to available methods[10].

H_2tfapd is a colourless solid, m.p. 142–143°C [from differential thermal analysis (DTA) in nitrogen, 138°C]. Found: C, 44.8; H, 4.5; N, 8.3%. Calculated for $C_{13}H_{16}F_6N_2O_2$: C, 45.1; H, 4.6; N, 8.1%. ^{1}H nuclear magnetic resonance spectroscopy (NMR) in C^2HCl_3: $-CH_3$, 2.04, 2.05 =CH–, 5.33, 5.36; bridge $-CH_2$ and –CH, 3.53 and 4.0, respectively; CH_3(bridge) 1.37; and NH 11.22 ppm.

Cu-tfapd is a violet solid, m.p. 206°C, Ni-tfapd is red-brown crystals, m.p. 245°C, Pd-tfapd is yellow crystals, m.p. 264°, and VO-tfapd is pink needles, m.p. 218–219°C.

Instrumentation

GC column phenomena were studied using a Packard-Becker Model 427 instrument equipped with a flame-ionization detector, or with a ^{63}Ni electron-capture detector (ECD) for the analytical work. Column evaluation was effected using glass coils (1.0–1.8 m long × 6.4 mm I.D.) filled with the various column packings prepared with 3–5% (w/w) loadings of the stationary phases SE-30, Dexsil 300GC, OV-3, OV-7, OV-17, OV-25, OV-210, Apiezon L, Kel-F wax and QF-1, and mixtures of Kel-F wax with SE-30 or OV-210. Supports examined included Chromosorb 750, Chromosorb W, Gas-Chrom Q, glass beads and Porasil (Type E). Analytical conditions represent the best overall conditions and involved using a glass column (1.8 m × 2 mm I.D.) packed with 3% (w/w) QF-1 on Chromosorb W (120–140 mesh, AW, DMCS treated, conditioned overnight at 250°C). Additional on-column silylation was not employed. The column temperature was 230°C and the detector and injection port temperatures were 250°C. High-purity nitrogen (18 ml/min) was used as the carrier gas and as the by-pass gas for the detector (20 ml/min).

Thermoanalytical data (thermogravimetry, TGA and DTA) were obtained using a Rigaku instrument (Thermoflex M8067) with 7.5-mg samples, in a stream of pure nitrogen (or air) flowing at 100 ml/min.

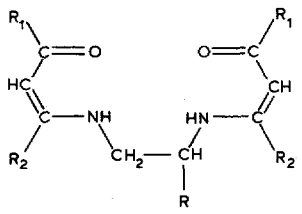
Analytical procedures

Duplicate samples of orchard leaves (280 mg, dried in a vacuum oven at 90°C for 24 h), reconstituted urine (10 ml) or water (10 ml) were subjected to complete oxidation in Kjeldahl flasks using a mixture (5–10 ml) of concentrated nitric and perchloric acids (5:2 by volume). The cool residue was quantitatively transferred into

a small beaker (50 ml) and the volume reduced nearly to dryness. Nine standards in the range 0–2.0 μg of copper and nickel were also acidified with the acid mixture (5 ml) and evaporated almost to dryness. All residues were then buffered with an ammonia solution (15 *M*, 5 ml), followed by the addition of a methanolic solution of the ligand (5 ml, 50 ppm) and gentle heating on a water-bath (with more ammonia, if necessary) to complete the chelation reaction. The cold solution was diluted with water (20 ml) and extracted with benzene. The combined extracts (10.0 ml), which are stable for several weeks, were usually chromatographed the next day. Aliquots (1 μl) were injected on to the column and from the chromatograms, peak areas (by triangulation) or heights were determined for the preparation of the calibration graphs and the analyses.

RESULTS AND DISCUSSION

As mentioned earlier, tetradentate Schiff bases of structure I have been used in the past for the analyses[7,8,11,12] of copper, nickel, palladium and vanadium. Depending on the metal ion, these chelates undergo[13,14] at least two oxidation reactions, introducing unsaturation and one or more keto groups in the ethane bridge. Interestingly, the dehydrogenated chelates (structure II) are more stable and often more volatile than the parent chelates[5]. Unfortunately, the preparation of the corresponding ligands has not been successful so far, nor has attempted on-column conversion been successful when a short bed (1–20 mm) of catalyst* was incorporated into the injection port end of glass columns that had resolved the parent from the dehydrogenated species.



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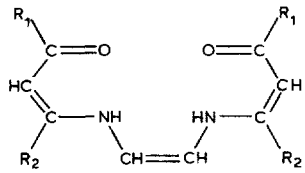


II

It has been noted[13,14] that among the Schiff base chelates of copper, nickel vanadium and palladium, the nickel chelates show the greatest tendency toward spontaneous oxidation. Further, the relative stability of nickel chelates containing alkyl, branched alkyl and fluoroalkyl substituents (where R is H and CH_3 in structure I) showed a close parallel between the TGA (and DTA) data in air and the oxidation potential in solution, as measured by cyclic voltammetry. The higher stability of the fluorinated chelates contrasts with the non-fluorinated chelates, whose higher volatility and wider volatility differences facilitate separation by GC. Other advantages of the non-fluorinated ligands include their ease of preparation and faster reaction in chelate formation. Nevertheless, from the practical viewpoint, sensitivity to the ECD

* As examples, palladium powder and palladized carbon (10% by weight) have been employed.

and the higher stabilities of the fluorinated chelates favour them as analytical reagents.

Among the possible fluorinated Schiff bases, H_2tfapd was chosen for the present study in order to eliminate problems[15] of the preparation and purification of a single isomeric compound, such as were observed with the related ligand derived from 1,1,1-trifluoro-5,5-dimethylhexane-2,4-dione and 1,2-diaminoethane (that is, structure I where R_1 is CF_3 and R_2 is C_4H_9). Despite the asymmetry in H_2tfapd (since $R_1 \neq R_2$), it can be conveniently prepared and purified to a stage of isomeric purity (where $R_1 = CF_3, R_2 = R = CH_3$), as was confirmed by GC and ^{1}H NMR spectroscopy. Of equal importance was the fact that the chromatographic resolution between Cu-tfapd and Ni-tfapd was significantly better than was possible with the cognate ligand (H_2tfaed) prepared with 1,2-diaminoethane (structure I, $R_1 = CF_3$, $R_2 = CH_3$ and $R = H$).

Column components

Based on the elution behaviour of the chelates, the liquid stationary phases examined in this work can be divided into two main groups. A third group, representing mixed liquid phases, is intermediate in properties in that each liquid phase exerts its own effect. Of the phases, SE-30, Dexsil 300GC, OV-3, OV-7, OV-17 and OV-25 belong to the first group where microgram amounts of chelates elute cleanly. Moreover, OV-3 columns clearly resolve VO-tfapd into two peaks, suggesting the presence of diastereoisomers[16,17]. Of the remaining stationary liquids, variable adverse behaviour was evident. The retention times of the chelates increase with increasing polarity of the stationary phases, as was demonstrated with the graded polarity of the OV series. Again, the retention time on QF-1 is longer, as expected, because of the fluorine-induced polarity in these chelates, whereas the corresponding non-fluorinated chelates exhibit shorter retention times[5].

With liquid phases of the first group, the copper or nickel chelates were well resolved from VO-tfapd but only partially separated from each other. This behaviour closely follows the volatility trends found by TGA (see Fig. 1). Improved resolution

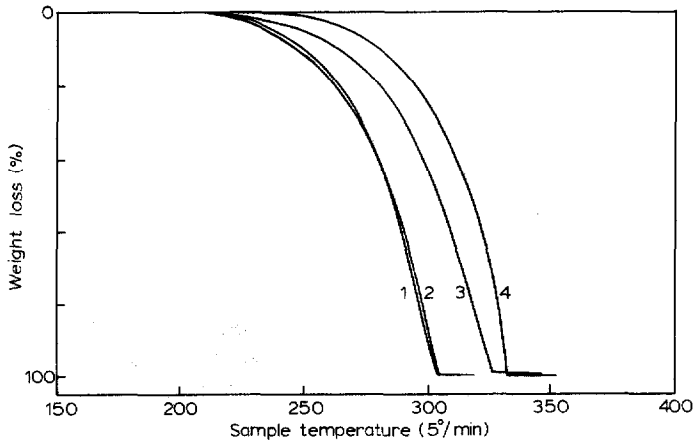
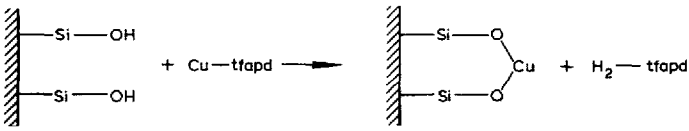


Fig. 1. Thermogravimetric curves for (1) Cu(II), (2) Ni(II), (3) VO(IV) and (4) Pd(II) derivatives of H_2tfapd.

can be obtained with more polar stationary phases such as QF-1 and, to a lesser extent, OV-210. However, the elution sequence is then reversed with the nickel chelate eluting ahead of the copper complex, as was also noted in earlier work[18].

As stated above, complicated column phenomena are observed with liquid phases of the second group. For example, on a Kel-F wax column, conditioned and operated between 200 and 300°C, decomposition of Cu-tfapd led to peak asymmetry and elution of a component whose retention time corresponded to the ligand. A possible explanation may be an exchange reaction involving surface silanol groups (depicted in Scheme 1) and is reminiscent of acid-catalysed hydrolysis of some related copper(II) chelates[19,20]. On this column, retention of Ni-tfapd was gradually reduced following successive injections, and was not altered by on-column silanization. Cu-tfapd also behaved unsatisfactorily on QF-1 and Apiezon L columns, although this was less serious on the former column if it had previously been exposed to a large number of injections of several copper(II) and nickel(II) chelates of this or other Schiff bases.



Scheme 1.

Generally, Ni-tfapd and VO-tfapd appeared more stable than Cu-tfapd under the chromatographic conditions studied. On Apiezon L columns, Cu-tfapd eluted as unsymmetrical peaks with elevated baselines, as was noted previously[21] also, although this was contrary to even earlier observations[18] with H_2tfaed. Because this discrepancy may be dependent upon the Apiezon L, its purification (by chromatography in this work) is recommended[22] in order to overcome any batch-to-batch variation due to the presence of highly unsaturated compounds[22,23] and the possibility of their subsequent oxidation[24–27].

It can be argued that liquid phases may introduce various types of reactive sites in addition to those already present on the uncoated support. Consequently, the properties of a solid support may undergo changes because of adsorption of a liquid phase on to active sites, or the liquid phase may be altered by virtue of its adsorption on the solid[28]. This is illustrated by the fact that a distinct improvement in the chromatographic behaviour of the copper chelate on QF-1 was noted when Chromosorb 750 was replaced with either Chromosorb W or Gas-Chrom Q (80–100 mesh). However, this was unexpected in view of the stated[22] inertness and the necessity for Chromosorb 750 in earlier work[29] with vanadium chelates. In addition to the absence of decomposition in this instance, resolution was also improved by using finer support particles (120–140 mesh) in narrow-bore glass columns (2 mm I.D.). No separation was achieved with glass bead or Porasil Type E surfaces under similar experimental conditions.

In coating the support, the best results were obtained by allowing the degassed suspension of the support to stand in a solution of QF-1 in ethyl acetate overnight (about 15 h). Solvent was removed by either the conventional dish-drying or fluidiza-

tion[30] techniques. Although the latter permits uniform coating to be achieved, only a fraction of the desired loading usually remains on the support.

Glass was clearly the preferred column material. With stainless-steel columns that were found to contain about 0.1 % copper, complete on-column conversion of nanogram amounts of ligand to the copper, and some nickel, chelate was observed. Problems such as on-column complexation of free ligands and catalysed decomposition of chelates within metal columns are, of course, well known[31–35] and may explain the high analytical results previously reported[11,12] in work with H_2tfaed.

Determination of copper and nickel

As the chromatographic behaviour of the three chelates of H_2tfapd complied with such requirements as stable baselines, good detector sensitivy and the absence of adsorptive loading, application of the ligand for analyses at the trace level were pursued. Its efficiency in the recovery of trace amounts of copper and nickel was established with concentrations of 100–400 pg, in increments of 100 pg. Depending on the method of processing the chromatographic data, the recoveries ranged from 95

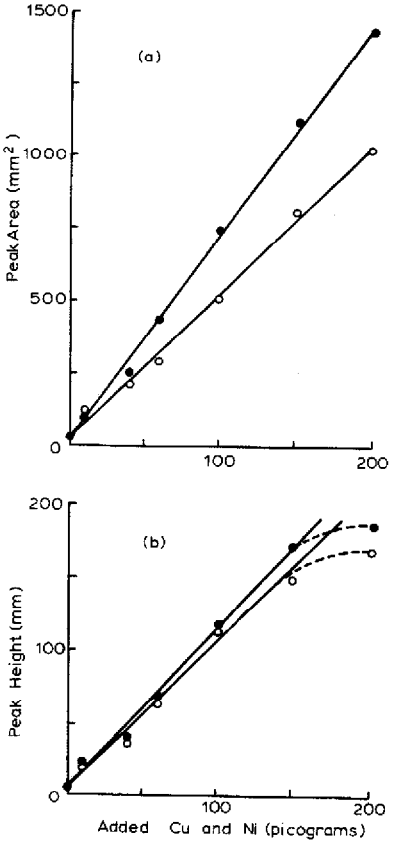


Fig. 2. Calibration graphs for the determination of copper (●) and nickel (○) by gas chromatography. Peak areas (a) and peak heights (b) are plotted against the equivalent amount of metal injected on to the column.

to 133% for copper and 75 to 105% for nickel using peak heights, and from 75 to 116% for copper and 73 to 91% for nickel when using peak area measurements. However, linear calibration graphs were obtained for concentrations up to about 200 pg (Fig. 2). Results for the analysis of three samples according to the method given under Experimental, including two materials of biological origin, are presented in Table I. The least satisfactory of these is undoubtedly the result obtained for copper in urine; otherwise, as preliminary findings, we believe the results show considerable promise and the method to be deserving of further refinement.

TABLE I

RESULTS OF ANALYSIS FOR COPPER AND NICKEL BY GAS CHROMATOGRAPHY

Sample	*Copper*		*Nickel*	
	Peak area method	*Peak height method*	*Peak area method*	*Peak height method*
Orchard leaves*	8.0 ± 2.8	9.5 ± 2.9	0.93 ± 0.32	0.76 ± 0.23
Urine**	80 ± 28	85 ± 26	15 ± 5	11.0 ± 3.0
Tap water***	153 ± 50	167 ± 51	30 ± 10	25 ± 8

* Certified values for NBS Standard No. 1571: copper, 12 ± 1 ppm; nickel 1.3 ± 0.2 ppm.
** Certified values for Q-Pak Urine, Lot No. 0521U001A: copper 163 ± 32 ppm, only.
*** Result determined potentiometrically by Dr. C. Maitra was 152 ppb of copper.

Perhaps the most difficult problem is that caused by the presence of an excess of the ligand. This compound responds strongly in the electron-capture detector and was not removed by washing with base (such as sodium carbonate or 1 *M* sodium hydroxide solution), or by reaction with trivalent ions such as Fe(III), and, for these reasons, its concentration was held at about ten times that of the total metal concentration. The effectiveness of this measure can be judged by the chromatograms of actual sample (and other) extracts shown in Fig. 3. Another distinct source of error probably lies in the measurement of small volumes of extracts taken for injection on to the column. This was compounded by the fact that a suitable internal standard was not found among the numerous compounds examined. Unfortunately, the retention time of the corresponding palladium(II) chelate* was too long for the purpose and was also identical with that of the VO(IV) chelate.

Determination of vanadium

The occurrence of vanadium with copper and nickel in many sample materials of interest[21] and the selectivity and sensitivity readily attainable in quantifying these elements with Schiff bases have been discussed elsewhere[4]. The added advantage of the fluorinated compound H_2tfapd in providing still greater detector sensitivity was, however, not realized because of the poor conversion of vanadium to VO-tfapd at the microgram level. Indeed, not only is the reaction with this fluorinated ligand difficult

* The retention time of the palladium(II) chelate is about twice as long as the copper(II) chelate (see Fig. 3). Large halogenated organic molecules, such as DDT, have retention times appreciably shorter than those of the copper and nickel chelates.

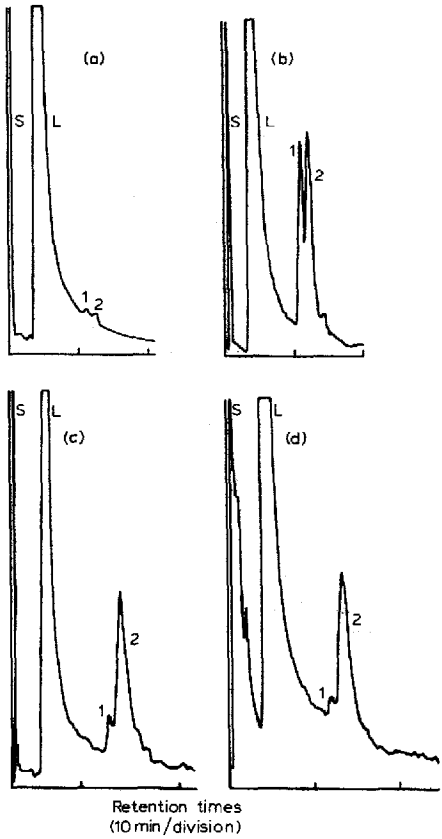


Fig. 3. Electron-capture detector response for Cu-tfapd and Ni-tfapd in extracts from (a) a reagent "blank", (b) standards (peaks correspond to 100 pg of each metal), (c) orchard leaves and (d) urine samples. Peaks: 1, Cu chelate; 2, Ni chelate; S, solvent; L, ligand.

but, upon extended exposure at room temperatures to sulphurous acid, which is very effective[4] for the reduction of V(V) to V(IV), slow decomposition of the copper and nickel chelates also occurs.

At this stage, therefore, it appears that the reagent H_2tfapd is limited to the sensitive, simultaneous determination of copper and nickel.

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