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Note

High-performance liquid chromatography of carbonyl compounds as 2,4-dinitrophenylhydrazones with electrochemical detection

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The increasing acidity of atmospheric cloud, fog and precipitation is of environmental concern. It has long been recognized that the major acids responsible for "acid rain", sulphuric and nitric acids, are formed in the troposphere from sulphur dioxide and oxides of nitrogen (NO, NO_2), respectively, by various chemical reactions. Recent studies[1,2] have taken into account the important role of some organic compounds, such as aldehydes and ketones, in such chemical reactions, and the development of sensitive methods for the determination of aldehydes and other carbonyls is important for the elucidation of the processes that control the acidity and composition of atmospheric precipitation.

Grosjean and co-workers[3,4] studied the possibility of using high-performance liquid chromatography (HPLC) for the separation and quantitation of nanogram amounts of some carbonyl compounds considered ubiquitous in the air. The method is based on the separation of the carbonyl compounds as 2,4-dinitrophenylhydrazone (DNPH) derivatives using isocratic elution and quantitation with ultraviolet detection at 360 nm. HPLC with electrochemical detection has been applied with success to picomole levels of readily oxidizable species such as aromatic amines and phenols[5–12].This work shows the analytical possibilities of such a detection method for DNPH derivatives in future applications in the analysis of carbonyl compounds in acid rain.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 1010 A chromatograph, modified with a Rheodyne 7120 sample injector and a 20-μl loop, was used. A commercial reversed-phase column Erbasil C_{18}, 10 μm (250 × 4.6 mm I.D.), was employed for all measurements.

Detection was achieved with a Metrohm 656 electrochemical detector equipped with a glassy carbon electrode. The surface of the electrode was renewed each day by mechanical polishing using alumina powder (0.3 μm). A Metrohm VA 641 instrument was employed as a potentiostat and d.c. amplifier. The eluent was water (triply distilled)–methanol (HPLC grade) (30:70) containing an electrolyte (1 g/l $LiClO_4$ and 0.05 g/l H_2SO_4).

Reagents

The 2,4-dinitrophenylhydrazine reagent and the carbonyl compounds were commercial reagent-grade samples (Carlo Erba) and were not further purified.

The standard 2,4-dinitrophenylhydrazones were synthesized by the well known reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine in the presence of an acid that promotes the protonation of the carbonyl group. The reaction products were purified by recrystallization from ethanol. Standard solutions were prepared by dissolving weighed amounts of the pure hydrazones in dichloromethane. Portions of these solutions were then diluted with the eluent to give the test solutions.

RESULTS AND DISCUSSION

Fig. 1. shows a chromatogram of nine carbonyl compounds examined as DNPH derivatives. Table I summarizes the retention data of the derivatives and the derivatizing reagent on an Erbasil C_{18} column.

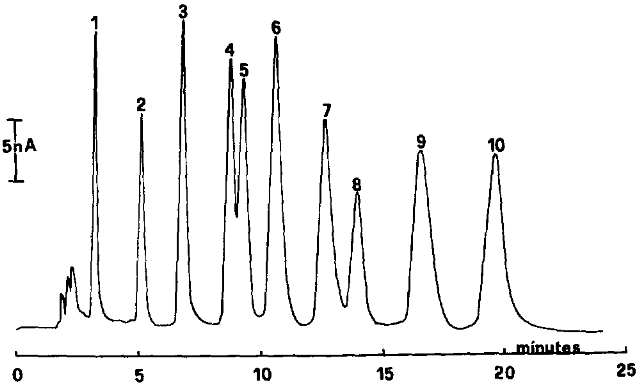

Fig. 1. HPLC separation of carbonyl compounds as DNPH derivatives. 1 = Reagent; 2 = methanal; 3 = ethanal; 4 = propenal; 5 = propanal; 6 = acetone; 7 = *n*-butanal; 8 = 2-butenal; 9 = 3-methylbutanal; 10 = benzaldehyde. Column, Erbasil C_{18}; eluent, methanol–water (70:30) containing 1 g/l $LiClO_4$ and 0.05 g/l H_2SO_4; flow-rate 1.4 ml/min; detector, 1.10 V.

All the compounds, were separated under the reported analytical conditions except for the partial overlap of the isobutanal and *n*-butanal derivatives. The derivatizing reagent was eluted shortly after the void volume and clearly before the formaldehyde derivative.

The electrochemical behaviour of the DNPH derivatives was examined by measuring the electrochemical detector responses (peak heights) at different working electrode potentials. The results, shown in Figs. 2 and 3, indicate that there was no significant signal at values lower than +0.80 V and the optimal potential was about +1.10 V. A comparison of the curves shows a relatively higher response of unsaturated aldehyde derivatives at low potentials.

A level of five times the signal-to-noise ratio was used to estimate the detection limits for the carbonyl derivatives, and these are reported in Table II. The detection limits vary from 30 pg for methanal to 212 pg for benzaldehyde.

TABLE I

HPLC RETENTION VALUES (CAPACITY FACTORS, k') FOR 2,4-DINITROPHENYLHYDRAZONES OF ALDEHYDES AND ACETONE

Elution conditions: methanol-water (70:30) containing 1 g/l $LiClO_4$ and 0.05 g/l H_2SO_4; flow-rate, 1,4 ml/min; electrode potential, 1.10 V *vs.* Ag/AgCl.

Compound	k'
2,4-Dinitrophenylhydrazine	0.43
Methanal	1.58
Ethanal	2.54
Propenal	3.45
Propanal	3.69
Acetone	4.26
Isobutanal	5.20
n-Butanal	5.37
2-Butenal	5.95
3-Methylbutanal	7.33
Benzaldehyde	8.80

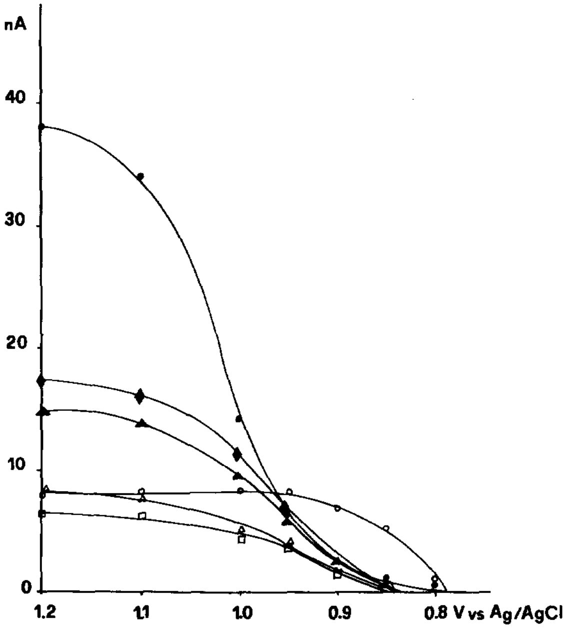

Fig. 2. Relationship between detector response and applied potential for 200 pmole of DNPH derivatives: ●, methanal; ◆, ethanal; ▲, propanal; ○, 2-butenal; △, 3-methylbutanal; □, *n*-butanal.

Fig. 4 shows some calibration graphs obtained at a potential of + 1.10 V. In the concentration range studied the relationship between concentration and detector response is clearly linear, except for methanal.

Fig. 5 shows a typical chromatogram obtained under sensitive conditions.

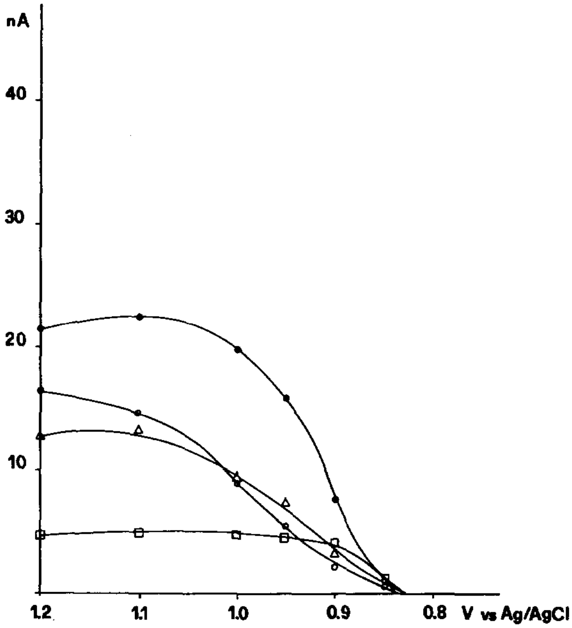

Fig. 3. As Fig. 2 for the following DNPH derivatives: ●, propenal; ○, isobutanal; △, acetone, □, benzaldehyde.

To conclude, the use of an electrochemical detector for the trace determination of carbonyl compounds as DNPH derivatives offers good prospects. The extension of the method to environmental samples such as rain or fog requires accurate studies of the derivatization step and all possible oxidizable interferences. Work is in progress in our laboratory in this direction.

TABLE II

DETECTION LIMITS AT AN OXIDATION POTENTIAL OF + 1.10 V

Peak height 5 × noise at 10 nA full-scale.

Carbonyl compounds as DNPH derivatives	*Detection limit (pg)*
Methanal	30
Ethanal	44
Propenal	56
Propanal	58
Acetone	58
Isobutanal	72
n-Butenal	72
2-Butenal	140
3-Methylbutanal	172
Benzaldehyde	212

* Referred to underivatized carbonyl compounds.

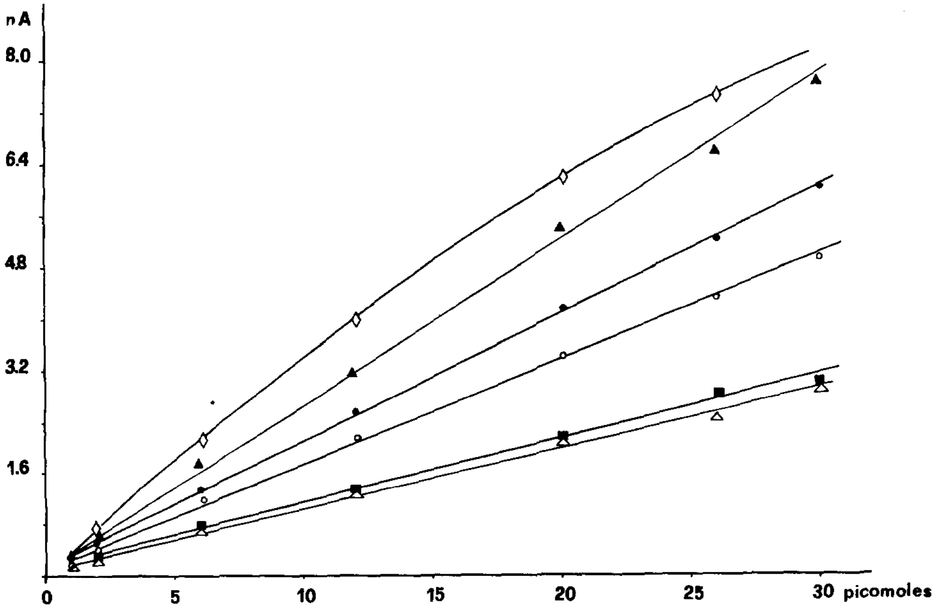

Fig. 4. Calibration graphs at a potential of + 1.10 V for DNPH derivatives: ◇, methanal; ●, ethanal; ○, propanal; ■, 2-butenal; △, *n*-butanal; ▲, propenal.

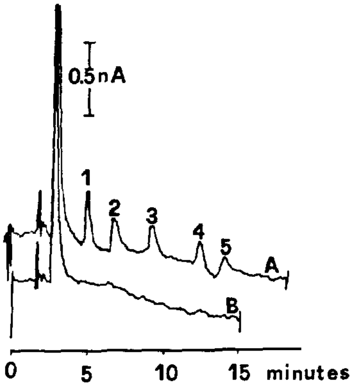

Fig. 5. Chromatograms of (A) DNPH derivatives of 1, methanal; 2, ethanal; 3, propanal; 4, isobutanal; 5, 2-butenal (2 pmole of each component); (B) blank. Chromatographic conditions as in Fig. 1.

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