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(i) solvent selection
(ii) column selection
(iii) selection of isocratic or gradient conditions
(iv) selection of detection method (detector, λ etc.)
(v) combinations of columns and solvents

This procedure is very time consuming and cannot be carried out unattended. Consequently, most users do not optimise their separations.
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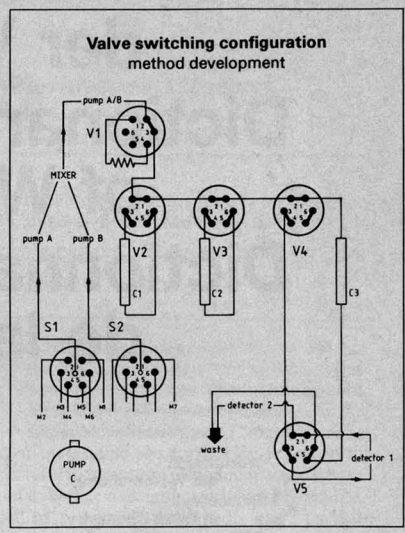
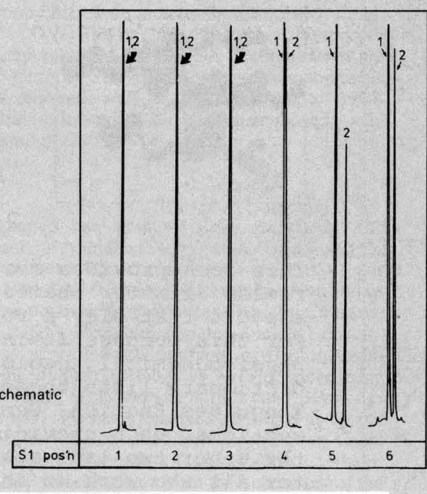


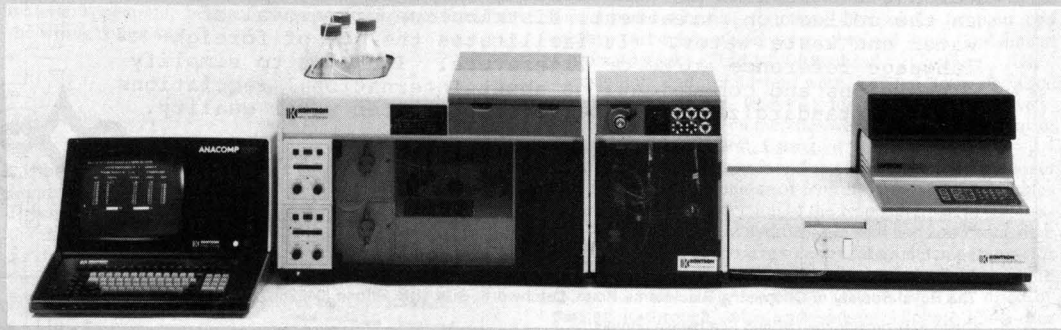
SOLVENT SELECTION – CHLOROANILINES

The logical approach to reversed phase separation is to progress
from (i) aqueous solvent systems (M1) to
(ii) ion-suppression solvent systems (M2–M4) to
(iii) ion-pairing solvent systems (M5, M6)

Each of these systems can be used together with an organic mobile phase such as methanol, acetonitrile or binary mixtures of the solvents to build up ternary or multi-solvent eluents.
The sample chosen demonstrates the MAJOR role played by the aqueous solvents in achieving chromatographic separation of two chloroanilines under isocratic conditions. Using this configuration, one of the 3 identical columns can be dedicated to each of the 3 modes of separation (i–iii), thus reducing equilibration time.

Conditions:
see valve switching schematic
(e.g. pos. 1 = M1)





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Organophosphorus Sulphides, Sulphoxides and Sulphones Part 2.* Characterisation by Gas Chromatography - Mass Spectrometry

John P. G. Wilkins, Alan R. C. Hill and Donald F. Lee
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The gas-chromatographic behaviour and electron-impact mass spectrometric characteristics are reported for nearly 90 organophosphorus sulphides, sulphoxides and sulphones, used as or derived from pesticides.

Keywords: *Organophosphorus pesticides; metabolites and contaminants; residues analysis; gas chromatography; electron-impact mass spectrometry*

Organophosphorus sulphides, sulphoxides and sulphones are widely used as agricultural pesticides, despite their generally high toxicity to man. Residual levels of these pesticides and, where appropriate, their oxidation or reduction products, must consequently be very low in harvested crops and derived foodstuffs to ensure consumer safety. Determination of these residues is usually performed by gas chromatography (GC), exploiting the high sensitivity and relatively specific response of currently available phosphorus detectors. However, as described in Part 1,[1] the lability of many of these compounds requires that gas-chromatographic results must be interpreted with caution and, where possible, be supported by mass spectrometric (MS) evidence.

In support of a programme for surveying pesticide residues in UK foodstuffs, and also as part of a PhD project for one of us (J.P.G.W.), we have studied the gas-chromatographic behaviour and electron-impact and chemical ionisation (EI and CI) magnetic-sector mass spectrometric characteristics of nearly 90 organophosphorus sulphides, sulphoxides and sulphones used as, or derived from, pesticides, and present here a summary of data useful in their identification. Gas-chromatographic and mass spectral data are also given for some phosphorus-containing contaminants observed during this work.

Mass spectral information on 45 members of this group of compounds has been published[2-12] but little of this has yet been incorporated into the libraries of mass spectrometer data systems. Mass spectra for many of the toxic metabolites of these compounds have not been published and it can be difficult for the analyst to acquire analytical standards of them. We have therefore included as many of these pesticides and their toxic metabolites as were available, or could readily be prepared. We have included some appropriate corrections/additions to published mass spectral data where the published material appeared to be incomplete, confusing or assigned to the wrong compound.

Experimental

Apparatus

Three different gas chromatograph - mass spectrometer combinations were used for this work: Varian 1400 - VG Micromass 12B; Dani 3800 - VG Analytical 7035: and Hewlett-Packard 5790 - JEOL DX300. For those compounds that decomposed completely under the gas-chromatographic conditions used, spectra were obtained by direct insertion of the pure compounds. All the spectra reported here were produced by EI with an ionisation energy of 30 or 70 eV (mostly at 30 eV) and a source temperature of 150–200 °C, acquiring ions over the range *m/z* 20–620. In those situations where convincing relative molecular mass information was not provided by EI, CI, using 2-methylpropane or ammonia as the reagent gas, was employed. In addition, accurate mass measurement and/or metastable ion correlation was used to help identify apparently important fragment ions whose formation appeared to be due to complicated rearrangements. GC was performed as previously described,[1] using packed columns at temperatures from 150 to 240 °C, except that OV-1701 was used instead of OV-17 as the stationary phase. Relative retention times were measured on a 0.5 m × 2 mm column of 7% OV-1701 on 100–120-mesh Chromosorb W(HP), at a temperature of 220 °C, with a helium carrier gas flow-rate of 30 ml min^{-1}. When better gas-chromatographic resolution was required, a 25 m × 0.2 mm CP-Sil 19CB capillary column (Chrompak Ltd.) was employed, with splitless injection, on the HP 5790.

Chemicals

The pesticide names used here are those quoted in "The Pesticide Manual."[13] Aphidan (*S*-ethylsulphinylmethyl *O,O*-diisopropyl phosphorodithioate, also known as IPSP) was obtained from Berk Ltd. (London). Carbophenothion and its metabolites were obtained from Stauffer Chemical Company (Westport, CT, USA). Demeton, demeton-S-methyl, disulfoton, fenamiphos, fensulfothion, fenthion, oxydeprofos, sulprofos and some of their metabolites were obtained from Bayer UK Ltd. (Bury St. Edmunds, Suffolk) and Bayer AG (Leverkusen, FRG). Phorate, temephos, terbufos and some of their metabolites were obtained from Cyanamid of Great Britain Ltd (Gosport, Hampshire). Chlorthiophos, ethion, sulfotep and TEPP were obtained from the Laboratory of the Government Chemist (London). Bensulide, famphur and methyl carbophenothion were obtained from Greyhound Chromatography Ltd. (Birkenhead, Cheshire, UK). Demephion and thiometon were isolated from Pyracide (BASF) and Ekatin (Sandoz) formulations, respectively. Vamidothion and its metabolites were obtained from May & Baker Ltd. (Brentwood, Essex).

Aphidan sulphide was found as a contaminant in the parent sulphoxide. The sulphides of fensulfothion and oxydeprofos were prepared by reduction of the respective parent sulphoxides with concentrated hydrochloric acid and solid potassium iodide, in glacial acetic acid solution, at room temperature for 2–5 min. After dilution with water, the sulphides were extracted with dichloromethane. The extract was dried by passing it through anhydrous sodium sulphate and, after addition of toluene (to assist removal of the acetic acid) and heptane (to assist removal of the iodine generated during the reaction), the solvent was removed using a rotary evaporator.

* For Part 1 of this series, see reference 1.

A similar method of extraction was employed after the oxidations given below, with the addition of toluene where acetic acid was used. The sulphoxides of chlorthiophos, demephion, demeton, sulprofos and thiometon were prepared by oxidation with hydrogen peroxide (100 volume) in glacial acetic acid containing a trace amount of concentrated sulphuric acid, at room temperature for 10–15 min. The oxon sulphones of Aphidan, chlorthiophos, demephion, sulprofos and the oxon of famphur were prepared in a similar manner to the sulphoxides but with the reaction carried out at 40–80 °C for 10–20 min. Aphidan oxon sulphide was observed as a contaminant in the oxon sulphone preparation, presumably arising from oxidation of the Aphidan sulphide. The sulphones of chlorthiophos, demephion, sulprofos and thiometon were prepared from their respective sulphides, and those of Aphidan, oxydeprofos and temephos were prepared from their respective sulphoxides, by oxidation with potassium permanganate, using a method similar to that employed for residue determination[1] (except that the oxidant concentration was 1% *m/V*). Although the majority of the sulphones were produced in 10–30 min at room temperature, those of chlorthiophos and temephos required 30–60 min at 80 °C. In a few instances further purification of the products was required, and this was achieved by column chromatography using silica gel (Merck Art. 7734, Kieselgel 60) eluted with mixtures of hexane - acetone appropriate to the polarity of the product required.

Results and Discussion

Using similar experimental conditions (and an ionisation energy of either 30 or 70 eV) on our three mass spectrometers, the spectra produced from a given compound were almost identical.

There is a lack of uniformity in the presentation of mass spectral data in the literature and, where analogue spectra are given, the inaccuracies in printing can make the assignment of nominal mass difficult. Many of the spectra previously reported were generated using quadrupole mass spectrometers (as opposed to magnetic-sector instruments), which tended to discriminate against high mass ions. In spite of this, there is a good level of agreement between the spectra obtained and the majority of those which have already been published.[2–12] In those instances where differences are present, these generally appear to be due to exaggerated relative intensities of the low mass ions in the previously published spectra. Another source of disparity is that some workers have reported spectral data below *m/z* 20. We did not monitor ions below this value because of the critical dependence of their relative intensities upon operational conditions and their generally high background levels, which make it difficult to generate reproducible, uncontaminated spectra. We have found that enhanced production of high mass ions, which are generally more useful in characterising compounds, can be effected by the use of a lower source temperature (which, however, may cause problems due to source contamination and loss of chromatographic efficiency) and a lower ionisation energy (which can result in reduced sensitivity). Mass spectral data, presented in the format used in the "Eight Peak Index of Mass Spectra"[2] (EPI), recorded for the compounds either eluting as gas chromatographic peaks or from the direct insertion of the pure compounds, are given in Table 1. Reference materials of the organophosphorus sulphides, sulphoxides and sulphones may contain organophosphorus contaminants that can mislead the analyst (especially if the contaminant exhibits a shorter retention time and/or a better chromatographic peak than the desired compound) and therefore we have included mass spectral data on some of these compounds at the end of the table.

The sulphides of Aphidan, fensulfothion and oxydeprofos were included because biological reduction[14,15] of these pesticides, which are sulphoxides, could result in the sulphides appearing in residues.

In contrast to Ripley and Braun,[16] we were unable to obtain a GC peak corresponding to bensulide, but observed several related compounds apparently produced by pyrolysis. The spectrum that we recorded for bensulide (by direct insertion), is in marked contrast to that presented in EPI (Q3918), which exhibits a preponderance of low mass ions, possibly for the reasons outlined above. Our spectrum of carbophenothion is similar to those of Damico,[3] Lovins,[4] Mestres *et al.*[5] and Stan *et al.*[6] However, Mestres *et al.* reported a significant fragment ion at *m/z* 108 (which they claimed to be characteristic of both ethyl thiophosphates and ethyl dithiophosphates, a claim that we dispute), which we observed only as a minor component of the spectrum, and which is probably due to $(C_6H_4S)^+$. Support for this suggestion comes from the appearance of this ion as a significant component of the spectra of bis(4-chlorophenyl) disulphide and bis(4-chlorophenylthio)-methane (both found in technical grade carbophenothion). Stan *et al.*[6] quoted a characteristic ion at *m/z* 172, which we consider should be at *m/z* 171. Of the three spectra represented in EPI, one (Q4128) is markedly different from that obtained by us, no ions outside the range *m/z* 127–173 being reported. The spectra represented in EPI for carbophenothion metabolites are in good agreement with those obtained by us.

Chlorthiophos I (using the isomer assignment given by Worthing and Walker[13]) is the only isomer represented in EPI but, again, the spectrum has a preponderance of low mass ions. The three isomers of chlorthiophos, and their metabolites, present an interesting complexity. In each group of spectra produced for the sulphides, sulphoxides and sulphones, respectively, two were very similar whereas the third was markedly different. Liquid chromatographic separation of the isomers of the sulphide and their subsequent conversion into the sulphoxides and sulphones, followed by GC - MS examination, indicated that the singular spectra were due to chlorthiophos II and its metabolites. A preliminary study by proton and carbon-13 nuclear magnetic resonance spectroscopy (NMR) did not assist in the elucidation of the structure of the isomers.

The spectrum we recorded for demephion is very different from that given by Stan *et al.*[6] EPI contains eight spectra of demeton and its metabolites, which are in general agreement with our spectra, except for one (Q0295) of the three given for demeton-O, and that given for demeton-O sulphone (R0915). Our spectra of demeton-S-methyl, its sulphoxide and sulphone are similar to those of Stan *et al.*,[6] Skinner and Greenhalgh[7] and EPI, except that the spectrum recorded by Stan *et al.*[6] for the sulphoxide appears to represent a pyrolysis product (which is, in part, the subject of another report we currently have in preparation) and one (R6601) of the spectra of demeton-S-methyl sulphone presented in EPI is misleading in that no ions that we consider to be characteristic of this compound are given. The disulfoton spectra given by Damico[3] and Hattori *et al.*[8] agree with ours but those given by Stan *et al.*[6] and Mestres *et al.*[5] show some disparity, including an apparent lack of ions that we found in high abundance (*m/z* 60, 61, 88 and 89). Of the spectra of disulfoton and its metabolites presented in EPI, only one, of the sulphide (Q0301), shows a significant discrepancy from those that we present.

Our spectrum of ethion agrees with those of Damico,[3] Stan *et al.*[6] and Mestres *et al.*[5] (except that in reference 5 the ion at *m/z* 231 appears to have been misprinted as *m/z* 281). One (Q2111) of the two spectra given for ethion in EPI is in close agreement with our spectrum. The spectra of famphur and its oxon represented in EPI are in good agreement with our spectra. Our spectra of fenamiphos and its sulphoxide agreed very closely with those of Skinner and Greenhalgh[7] (although, owing to inadequate resolution in the printing of their spectra, some of the ions in these and other spectra appear to be one

Table 1. Mass spectral data for organophosphorus compounds

Compound name	Empirical formula	Molecular weight*	Mass to charge ratios								Relative intensities of most abundant ions								M^+	EPI† reference number
Aphidan sulphide	$C_9H_{21}O_2S_3P_1$	288	75	43	107	47	41	98	288	140	100	24	19	13	10	9	8	8	8	—
Aphidan	$C_9H_{21}O_3S_3P_1$	304	139	43	41	97	27	78	45	47	100	68	31	26	18	18	17	17	—	—
Aphidan sulphone	$C_9H_{21}O_4S_3P_1$	320	143	139	43	159	41	97	47	77	100	94	59	32	25	24	23	22	5	—
Aphidan oxon sulphide	$C_9H_{21}O_3S_2P_1$	272	75	74	43	41	128	115	47	187	100	82	48	28	22	22	22	17	10	—
Aphidan oxon sulphone	$C_9H_{21}O_5S_2P_1$	304	169	127	43	123	211	59	41	115	100	74	52	50	30	29	21	21	0.1	—
Bensulide†	$C_{14}H_{24}O_4N_1S_3P_1$	397	215	131	172	77	214	173	141	170	100	72	70	70	66	58	54	53	—	Q3918
Carbophenothion	$C_{11}H_{16}O_2Cl_1S_3P_1$	342	157	121	153	342	159	199	125	97	100	51	45	43	38	37	37	37	43	Q4123, Q4124, Q4128
Carbophenothion sulphoxide	$C_{11}H_{16}O_3Cl_1S_3P_1$	358	199	153	125	97	109	171	201	200	100	98	66	62	17	13	10	8	—	R6739
Carbophenothion sulphone	$C_{11}H_{16}O_4Cl_1S_3P_1$	374	153	199	125	97	159	171	201	185	100	87	52	34	17	15	9	8	5	R6348
Carbophenothion oxon	$C_{11}H_{16}O_3Cl_1S_2P_1$	326	183	109	326	139	157	111	155	75	100	95	55	53	44	44	42	40	55	R2907
Carbophenothion oxon sulphoxide	$C_{11}H_{16}O_4Cl_1S_2P_1$	342	109	183	139	81	137	75	155	127	100	61	32	31	29	27	21	15	—	—
Carbophenothion oxon sulphone	$C_{11}H_{16}O_5Cl_1S_2P_1$	358	183	109	139	137	75	155	127	184	100	45	30	23	19	18	9	7	—	R6350
Chlorthiophos I	$C_{11}H_{15}O_3Cl_2S_2P_1$	360	269	97	325	297	360	271	109	125	100	99	79	51	44	43	42	35	44	R9451
Chlorthiophos II	$C_{11}H_{15}O_3Cl_2S_2P_1$	360	222	97	224	257	45	360	289	259	100	97	77	61	45	43	41	40	43	—
Chlorthiophos III	$C_{11}H_{15}O_3Cl_2S_2P_1$	360	269	325	97	271	297	327	65	299	100	81	55	42	37	34	18	15	13	—
Chlorthiophos I sulphoxide	$C_{11}H_{15}O_4Cl_2S_2P_1$	376	97	341	125	285	343	109	153	313	100	85	56	53	36	33	32	32	0.2	—
Chlorthiophos II sulphoxide	$C_{11}H_{15}O_4Cl_2S_2P_1$	376	257	259	97	313	222	45	224	315	100	66	64	42	35	33	29	27	—	—
Chlorthiophos III sulphoxide	$C_{11}H_{15}O_4Cl_2S_2P_1$	376	97	341	269	325	271	343	285	297	100	86	80	58	40	37	36	34	1	—
Chlorthiophos I sulphone	$C_{11}H_{15}O_5Cl_2S_2P_1$	392	301	97	357	303	329	359	125	65	100	85	79	44	41	35	31	18	—	—
Chlorthiophos II sulphone	$C_{11}H_{15}O_5Cl_2S_2P_1$	392	257	259	97	313	315	285	287	179	100	64	49	43	35	25	22	22	—	—
Chlorthiophos III sulphone	$C_{11}H_{15}O_5Cl_2S_2P_1$	392	97	357	301	125	109	329	359	240	100	81	60	49	46	39	35	32	4	—
Demephion-O	$C_5H_{13}O_3S_2P_1$	216	74	75	143	41	76	125	47	109	100	33	9	7	6	5	4	3	0.5	—
Demephion-O sulphone	$C_5H_{13}O_5S_2P_1$	248	125	169	79	109	111	63	47	107	100	65	43	18	11	11	11	11	1	—
Demephion-O oxon sulphone	$C_5H_{13}O_6S_1P_1$	232	153	109	127	79	96	95	154	63	100	82	64	15	10	9	7	7	—	—
Demephion-S	$C_5H_{13}O_3S_2P_1$	216	74	142	112	75	109	41	76	79	100	15	13	12	10	9	8	7	3	—
Demephion-S sulphoxide‡	$C_5H_{13}O_4S_2P_1$	232	169	109	125	74	91	171	142	110	100	69	42	10	7	6	5	5	—	—
Demephion-S sulphone	$C_5H_{13}O_5S_2P_1$	248	169	109	125	168	110	142	170	127	100	52	48	32	21	20	10	8	0.5	—
Demeton-O	$C_8H_{19}O_3S_2P_1$	258	88	89	60	61	29	171	115	59	100	53	39	27	12	10	9	8	0.3	Q0292, Q0294, Q0295
Demeton-O sulphone	$C_8H_{19}O_5S_2P_1$	290	197	153	29	45	141	125	97	121	100	89	84	77	71	51	44	42	1	R0915
Demeton-O oxon sulphone	$C_8H_{19}O_6S_1P_1$	274	125	99	181	121	127	153	155	29	100	85	73	45	45	44	43	34	2	—
Demeton-S	$C_8H_{19}O_3S_2P_1$	258	88	60	29	89	61	114	115	93	100	42	14	13	13	10	9	9	0.8	P9503, P9504
Demeton-S sulphoxide‡	$C_8H_{19}O_4S_2P_1$	274	109	197	141	29	81	137	45	61	100	89	77	28	27	26	24	23	—	Q8208
Demeton-S sulphone	$C_8H_{19}O_5S_2P_1$	290	109	197	141	29	81	61	169	45	100	95	73	40	32	28	28	27	—	Q8207
Demeton-S-methyl	$C_6H_{15}O_3S_2P_1$	230	88	60	142	109	89	61	79	112	100	54	12	12	10	9	9	8	1	L0768, Q4536
Demeton-S-methyl sulphoxide‡	$C_6H_{15}O_4S_2P_1$	246	169	109	125	29	79	105	60	142	100	92	48	13	11	9	9	7	—	—
Demeton-S-methyl sulphone	$C_6H_{15}O_5S_2P_1$	262	169	109	125	110	168	142	29	170	100	51	33	15	13	9	8	7	—	R6601, R6602
Disulfoton	$C_8H_{19}O_2S_3P_1$	274	88	89	60	61	153	186	142	274	100	34	20	13	12	12	12	10	10	Q0296, Q0299, Q0301
Disulfoton sulphoxide‡	$C_8H_{19}O_3S_3P_1$	290	125	213	153	97	185	61	157	29	100	83	82	81	59	41	34	25	0.5	Q8210
Disulfoton sulphone	$C_8H_{19}O_4S_3P_1$	306	213	153	61	125	97	157	185	186	100	75	39	38	29	21	13	12	1	Q8209
Disulfoton oxon, see Demeton-S																				
Famphur	$C_{10}H_{16}O_5N_1S_2P_1$	325	218	93	125	217	44	109	219	220	100	29	24	21	14	11	11	6	1	P4602, P4603
Famphur oxon	$C_{10}H_{16}O_6N_1S_1P_1$	309	202	201	44	265	109	186	93	309	100	69	39	21	18	18	12	9	9	Q4867
Fenamiphos	$C_{13}H_{22}O_3N_1S_1P_1$	303	303	288	260	154	195	44	217	304	100	37	32	31	26	18	15	15	100	R9601
Fenamiphos sulphoxide	$C_{13}H_{22}O_4N_1S_1P_1$	319	304	122	303	319	154	196	80	44	100	84	35	29	29	25	24	21	29	S3424
Fenamiphos sulphone	$C_{13}H_{22}O_5N_1S_1P_1$	335	320	292	58	44	321	122	293	335	100	66	22	17	15	12	9	8	8	S3425

Table 1. *Continued*

Compound name	Empirical formula	Molecular weight*	Mass to charge ratios								Relative intensities of most abundant ions								M^+	EPI† reference number
Fensulfothion sulphide	$C_{11}H_{17}O_3S_2P_1$	292	292	156	140	97	125	109	29	139	100	62	59	47	28	28	21	21	100	—
Fensulfothion	$C_{11}H_{17}O_4S_2P_1$	308	293	308	141	97	125	153	109	292	100	55	49	47	47	33	33	19	55	P8887, P8888
Fensulfothion sulphone	$C_{11}H_{17}O_5S_2P_1$	324	324	109	97	188	125	172	157	170	100	99	83	67	46	35	30	25	100	—
Fensulfothion oxon sulphide	$C_{11}H_{17}O_4S_1P_1$	276	276	140	220	248	125	139	81	29	100	67	45	28	23	22	16	14	100	—
Fensulfothion oxon	$C_{11}H_{17}O_5S_1P_1$	292	277	141	109	249	292	221	278	81	100	63	42	33	25	25	20	17	25	—
Fensulfothion oxon sulphone	$C_{11}H_{17}O_6S_1P_1$	308	109	127	182	201	99	280	308	119	100	68	64	55	55	48	48	44	48	—
Fenthion	$C_{10}H_{15}O_3S_2P_1$	278	278	109	279	169	280	125	245	137	100	19	14	14	12	11	9	9	100	P4678, P4679
Fenthion sulphoxide	$C_{10}H_{15}O_4S_2P_1$	294	279	125	294	109	169	138	153	93	100	67	58	38	24	23	19	14	58	—
Fenthion sulphone	$C_{10}H_{15}O_5S_2P_1$	310	125	310	109	93	136	105	79	137	100	94	62	37	36	32	24	17	94	—
Fenthion oxon	$C_{10}H_{15}O_4S_1P_1$	262	262	247	263	264	135	217	109	215	100	31	20	10	10	9	8	6	100	R2356
Fenthion oxon sulphoxide	$C_{10}H_{15}O_5S_1P_1$	278	263	109	278	262	127	79	45	77	100	70	27	25	19	13	12	11	27	R2357
Fenthion oxon sulphone	$C_{10}H_{15}O_6S_1P_1$	294	294	215	104	109	231	230	295	279	100	60	28	23	19	19	18	17	100	R4948
Methyl carbophenothion	$C_9H_{12}O_2Cl_1S_3P_1$	314	157	125	45	93	159	314	316	171	100	46	37	36	35	19	9	9	19	—
Oxydeprofos sulphide	$C_7H_{17}O_3S_2P_1$	244	102	74	89	41	142	61	109	143	100	68	23	22	18	16	13	12	3	—
Oxydeprofos‡	$C_7H_{17}O_4S_2P_1$	260	183	41	125	109	29	102	143	74	100	55	34	29	16	14	13	12	0.2	—
Oxydeprofos sulphone	$C_7H_{17}O_5S_2P_1$	276	183	41	125	109	182	110	143	184	100	37	32	20	16	8	8	7	0.1	—
Phenkapton	$C_{11}H_{15}O_2Cl_2S_3P_1$	376	121	153	199	97	125	191	376	193	100	79	58	52	51	45	39	34	39	—
Phorate	$C_7H_{17}O_2S_3P_1$	260	75	121	97	93	47	260	65	29	100	25	12	11	10	9	9	6	9	Q0288, Q0290
Phorate sulphoxide	$C_7H_{17}O_3S_3P_1$	276	97	125	153	199	29	65	171	75	100	84	80	73	20	15	13	12	—	Q8375
Phorate sulphone	$C_7H_{17}O_4S_3P_1$	292	153	97	199	125	171	65	29	93	100	84	79	79	17	16	15	14	6	Q8376
Phorate oxon	$C_7H_{17}O_3S_2P_1$	244	74	75	171	111	138	109	47	244	100	72	43	28	17	16	15	13	13	—
Phorate oxon sulphoxide	$C_7H_{17}O_4S_2P_1$	260	109	183	75	137	155	139	81	127	100	46	37	27	26	26	22	21	—	Q8377
Phorate oxon sulphone	$C_7H_{17}O_5S_2P_1$	276	109	183	139	137	81	75	155	127	100	90	38	32	31	31	30	20	—	Q8378, Q8379
Sulprofos	$C_{12}H_{19}O_2S_3P_1$	322	322	156	140	139	113	43	155	125	100	80	59	50	28	26	24	21	100	—
Sulprofos sulphoxide	$C_{12}H_{19}O_3S_3P_1$	338	296	141	43	281	139	156	113	140	100	95	86	80	75	73	71	67	42	—
Sulprofos sulphone	$C_{12}H_{19}O_4S_3P_1$	354	188	312	43	113	172	141	125	155	100	70	41	31	28	21	17	16	15	—
Sulprofos oxon	$C_{12}H_{19}O_3S_2P_1$	306	140	306	139	43	125	156	307	97	100	90	35	21	18	16	14	14	90	—
Sulprofos oxon sulphoxide	$C_{12}H_{19}O_4S_2P_1$	322	307	139	167	43	97	141	125	140	100	77	50	39	33	32	31	22	21	—
Sulprofos oxon sulphone	$C_{12}H_{19}O_5S_2P_1$	338	172	196	43	139	296	188	97	157	100	61	56	51	37	36	36	28	15	—
Temephos‡	$C_{16}H_{20}O_6S_3P_2$	466	466	467	93	468	203	125	357	341	100	20	16	16	14	11	6	4	100	Q9302, Q9303, Q9304
Temephos sulphoxide‡	$C_{16}H_{20}O_7S_3P_2$	482	125	434	109	93	233	435	482	466	100	66	25	23	16	15	14	12	14	R6693
Temephos sulphone‡	$C_{16}H_{20}O_8S_3P_2$	498	203	125	498	109	93	388	265	499	100	67	51	48	40	14	14	12	51	—
Terbufos	$C_9H_{21}O_2S_3P_1$	288	57	231	103	153	186	142	97	288	100	71	31	18	12	11	10	10	10	R4175, R4176
Terbufos sulphoxide‡	$C_9H_{21}O_3S_3P_1$	304	57	97	153	29	125	41	199	186	100	83	57	49	47	38	36	29	—	—
Terbufos sulphone	$C_9H_{21}O_4S_3P_1$	320	153	199	57	125	97	264	200	172	100	62	57	56	36	33	25	24	5	—
Terbufos oxon	$C_9H_{21}O_3S_2P_1$	272	171	57	215	170	272	143	115	126	100	63	60	56	31	29	19	15	31	—
Terbufos oxon sulphoxide‡	$C_9H_{21}O_4S_2P_1$	288	170	232	109	183	57	139	41	137	100	47	35	27	20	17	17	16	—	—
Terbufos oxon sulphone	$C_9H_{21}O_5S_2P_1$	304	156	183	184	109	140	57	75	155	100	86	85	85	84	80	47	37	—	—
Thiometon	$C_6H_{15}O_2S_3P_1$	246	88	89	60	125	61	246	90	158	100	21	19	11	8	5	5	4	5	Q3525, Q3526
Thiometon sulphoxide‡	$C_6H_{15}O_3S_3P_1$	262	125	185	157	93	59	159	187	88	100	83	78	14	14	8	8	7	—	—
Thiometon sulphone	$C_6H_{15}O_4S_3P_1$	278	125	185	93	157	29	158	186	61	100	57	36	20	14	11	8	8	0.5	—
Thiometon oxon, see Demeton-S-methyl																				
Vamidothion	$C_8H_{18}O_4N_1S_2P_1$	287	87	145	146	142	109	88	58	60	100	46	18	16	15	14	12	9	1	Q7762
Vamidothion sulphoxide‡	$C_8H_{18}O_5N_1S_2P_1$	303	169	109	125	58	87	143	142	86	100	48	28	22	20	14	13	10	—	—

Table 1. *Continued*

Compound name	Empirical formula	Molecular weight*	Mass to charge ratios								Relative intensities of most abundant ions								M^+	EPI* reference number
Vamidothion sulphone	$C_8H_{18}O_6N_1S_2P_1$	319	87	169	109	125	58	110	86	142	100	34	19	18	8	7	7	6	—	—
O,*O*-Diethyl-*O*-phenyl phosphorothioate	$C_{10}H_{15}O_3S_1P_1$	246	94	246	110	109	97	105	141	190	100	78	63	43	26	20	17	17	78	—
O,*O*-Diethyl-*O*-(2,4,5-trichlorophenyl) phosphorothioate	$C_{10}H_{12}O_3Cl_3S_1P_1$	348	97	313	257	315	259	125	285	109	100	64	59	47	40	35	33	33	—	—
Ethion	$C_9H_{22}O_4S_4P_2$	384	231	153	121	125	384	97	233	199	100	50	35	24	22	15	14	13	22	Q2111, Q2112
Oxydeprofos sulphone isomer	$C_7H_{17}O_5S_2P_1$	276	183	41	125	109	142	143	29	79	100	49	37	18	17	14	11	9	0.1	—
Sulfotep	$C_8H_{20}O_5S_2P_2$	322	322	202	121	93	65	174	238	266	100	56	52	51	41	40	37	36	100	L0995, Q9666, Q9668
TEPP	$C_8H_{20}O_7P_2$	290	263	161	179	235	162	207	99	191	100	94	82	73	67	55	43	23	7	G0523, P8008, P8009
Tetraethyl pyrophosphorotrithioate	$C_8H_{20}O_4S_3P_2$	338	97	121	65	93	125	29	338	153	100	90	76	65	52	46	41	33	41	—

* Molecular weights are given according to the "Eight Peak Index"[2] format, in which molecular weights are calculated from the integral values of atomic weights of the most abundant isotope of each element present, not according to the usual definition of average relative molecular mass.

† Reference number of spectral data presented in the Eight Peak Index.[2]

‡ Spectra obtained by direct insertion of the pure compound.

mass unit different from the values that we have obtained), whereas those presented in EPI appear to have exaggerated abundances of low mass ions. The spectra of fensulfothion presented in EPI, and of fensulfothion and its oxon presented by Skinner and Greenhalgh,[7] agree closely with our data. Our spectrum of fenthion is similar to that of Stan *et al.*[6] and those of EPI but the oxon metabolites given in EPI show an exaggerated low-mass ion abundance. Our methyl carbophenothion spectrum is very similar to those given by Damico,[3] Stan *et al.*[6] and two of those presented in EPI, whereas the third EPI spectrum (Q4826) lacks the important molecular ion.

Our phenkapton spectrum compares well with two of the spectra in EPI whereas the third (Q7760) is in complete disagreement. Our spectrum of phorate agrees with those of Stan *et al.*,[6] Skinner and Greenhalgh[7] and Mestres *et al.*,[5] except that the last of these did not report a peak at *m/z* 75. Our spectra of phorate oxon and phorate sulphone agree with those of Skinner and Greenhalgh[7] but the spectrum that Singh and Cochrane[9] assigned to phorate oxon sulphoxide is identical with that which we obtained from phorate sulphoxide.

The spectra presented in EPI for phorate, and four of its metabolites, have ions in common with our spectra but several of them exhibit enhanced low mass ion abundances and thus, again, do not record the more characteristic high mass ions. Our sulfotep spectrum agrees with those of Damico,[3] Stan *et al.*[6] and EPI. Our TEPP spectrum is similar to that of Tatematsu *et al.*[10] and one of those (G0523) given in EPI, whereas the other two spectra given in the latter appear to represent triethyl phosphate, a commonly observed decomposition product of TEPP. The spectrum we obtained for temephos, by direct insertion, is not very different from that published by Biros and Ryan[11] and is similar to two (Q9302 and Q9304) of those given in EPI, but that given by Stan *et al.*[6] (obtained by GC), unlike our spectrum, has ions of high abundance at *m/z* 263, 344, 360 and 435. Our spectrum of temephos sulphoxide has a greater abundance of characteristic high mass ions than that reported in EPI. The spectra we observed for terbufos and its metabolites agree very closely with those reported by Wei and Felsot,[12] except that their spectrum of the oxon sulphone lacks ions at *m/z* 140, 156 and 184, which we consider to be important components of the spectrum. The EPI spectra for terbufos, and for thiometon, are very similar to our spectra. The spectrum of vamidothion that we report is similar to that presented in EPI but appears to differ considerably from that given by Mestres *et al.*[5]

The spectral data that we have reported, for the compounds listed in Table 1, appear to be consistent with the structures that have been published for them.

Chemical ionisation would usually be the preferred ionisation technique to confirm the presence of low concentrations of most of these compounds in extracts of biological samples, especially where relatively high concentrations of co-extracted lipids are also present, but there seems little point in our reporting such spectra here because they convey little extra information, other than the relative molecular mass, and the spectra produced are dependent upon source design, source temperature and reagent gas employed.

However, we found that the compounds that produced little, or no, detectable molecular ion peak (intensity less than 0.1% of base peak) under EI often gave a relatively more abundant (though still weak) protonated molecular species, $(M + H)^+$. Detection of such relatively low abundance ions required a high sample pressure in the ion source (produced by direct insertion of microgram amounts of sample) but did allow us to obtain molecular weight information for these compounds, under EI conditions.

We regard relative retention times as an important part of the characterisation of these compounds because, in addition to the possibility of generating artifacts from them by pyrolysis during GC, a few of them produced very similar mass spectra. Gas-chromatographic relative retention times are given in Tables 2 and 3.

Relative retention times may vary with temperature and the individual column used, but we found that the order of elution of these compounds was similar over a wide range of conditions, using packed or capillary columns.

We were unable to achieve gas-chromatographic elution of even a small proportion of the injected material of bensulide, temephos and its sulphoxide and sulphone, or of the sulphoxides of demephion-O, demephion-S, terbufos, terbufos oxon,

Table 2. Retention times of organophosphorus sulphides, sulphoxides and sulphones, relative to malathion (= 1.00)

	Sulphide	Sulphoxide	Sulphone
Aphidan	0.40	1.20	1.25
Aphidan oxon	0.30		1.00
Carbophenothion	3.40	8.50	7.90
Carbophenothion oxon	2.70	6.70	6.25
Chlorthiophos I	2.85	5.65	7.50
Chlorthiophos II	2.35	4.60	4.50
Chlorthiophos III	2.50	4.90	6.55
Demephion-O	0.20	*	0.70
Demephion-S	0.25	*	1.10
Demephion-O oxon			0.55
Demeton-O	0.30		1.00
Demeton-S	0.40	1.60*	1.65
Demeton-O oxon			0.85
Demeton-S-methyl	0.35	1.30*	1.35
Disulfoton	0.55	2.20*	2.45
Disulfoton oxon, see Demeton-S			
Famphur			4.40
Famphur oxon			3.75
Fenamiphos	2.05	7.10	7.30
Fensulfothion	1.15	3.50	4.00
Fensulfothion oxon	0.95	3.00	3.35
Fenthion	1.15	3.80	4.05
Fenthion oxon	1.00	3.30	3.45
Methyl carbophenothion	2.85		
Oxydeprofos	0.35	1.15*	1.35
Phenkapton	5.70		
Phorate	0.30	1.20	1.30
Phorate oxon	0.25	0.85	0.95
Sulprofos	3.40	10.7	11.7
Sulprofos oxon	2.70	8.00	9.00
Terbufos	0.30	*	1.50
Terbufos oxon	0.25	*	1.25
Thiometon	0.40	*	1.80
Thiometon oxon, see Demeton-S-methyl			
Vamidothion	2.50	*	5.35*

* Largely or wholly decomposed during injection and/or chromatography.

Table 3. Retention times of some organophosphorus contaminants observed in pesticide standards, relative to malathion (= 1.00)

Contaminant	Relative retention
O,O-Diethyl-*O*-phenyl phosphorothioate, found in fensulfothion	0.25
O,O-Diethyl-*O*-(2,4,5-trichlorophenyl) phosphorothioate, found in chlorthiophos	0.95
Ethion, found in terbufos sulphoxide	2.95
Oxydeprofos sulphone isomer, by-product of oxidation of oxydeprofos	1.15
Sulfotep, found in technical demeton	0.35
TEPP, found in oxidised technical demeton	0.25
O,O,O,O-Tetraethyl pyrophosphorotrithioate, found in phorate sulphone	0.85

thiometon and vamidothion. Coupled liquid chromatography - mass spectrometry would appear to offer possible advantages (because of the obvious instability of some of these compounds during gas chromatography) but we have had very little experience of this. For most of the labile sulphoxides, it is convenient to convert them into the sulphones,[1] which are more stable. Temephos and its metabolites may be detected in some biological extracts using a direct-insertion probe (at temperatures of up to 400 °C), exploiting the very low volatility and the relative abundance and high mass of their molecular ions. However, for the confirmation of trace levels of bensulide, vamidothion sulphoxide and its sulphone, liquid chromatography may be the only satisfactory means of introduction into the mass spectrometer.

Nonetheless, we find that the identity and concentration of the majority of these compounds, occurring as residues, can be readily confirmed using GC - MS.

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Inter-laboratory Calibration for the Quality Control of Pesticide Analysis (1982–1983)

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The organisation and running of six inter-laboratory exercises in South Africa in 1982 and 1983 are described. Analyses were carried out on solutions of pesticides, on residues in orange and cabbage pulp samples, peanut butter and preserved cream, and two formulations. The results indicated that most laboratories performed well but that some should strive to improve their analytical capabilities.

Keywords: *Inter-laboratory calibration; pesticide analysis; formulation analysis; collaborative studies; pesticide residues*

In a previous publication[1] the organisation of a quality control programme for pesticide analysis in South Africa was described. The programme consisted of a number of inter-laboratory calibration exercises (ICEs) and work on a solution of pesticides and three foodstuff samples containing residues in the period up to the end of 1981 was reported. It was envisaged that the programme would continue and that a larger number of laboratories would become involved. However, this proved to be difficult to achieve because pesticide analysis is not centrally funded and participation is not mandatory. Nevertheless, the Task Group for ICEs continued with its efforts and during 1982 and 1983 there was a slow gain in the number of participating laboratories.

A number of laboratories in South Africa are mainly concerned with formulation analysis and, because they also expressed the need for analytical quality control, it was decided to include at least one formulation analysis in the annual programme. As the preparation and testing of ICE samples and the running of each exercise is time consuming it was decided to limit the programme to three exercises per year. The exercises were again planned so that each laboratory could participate in at least one ICE per year. In this paper the ICEs undertaken in 1982 and 1983 are described and the results reported.

Procedure

Programme

The samples for the 1982 and 1983 programmes included one formulation, a solution containing two pesticides combined with a vegetable or fruit matrix containing the same two pesticides and one fatty matrix. Most of the samples contained two pesticides of which the identity of only one was known. Reference standards were supplied for both, with the unknown marked X. All laboratories were asked to compare the reference standards with their own and to submit results of this comparison to the Task Group, which would then be in a position to evaluate the quality of standards used in routine pesticide analysis.

Each participating laboratory was given a month in which to complete the ICE and submit its results to the Task Group. This in effect meant that results were received back two months after the start of an ICE. It was decided to permit up to two analysts per laboratory to participate in a specific ICE, provided that the supervisor ensured that these analysts worked independently. Each analyst was allocated a code number known only to the Task Group and each analyst was requested to submit a detailed report on his dilutions and calculations. Only one value per analyst was, however, considered in the evaluation of the results.

ICE 1/82

The sample consisted of 100 ml of suspension concentrate containing atrazine with simazine as the unknown. The participant was informed that the unknown compound was an *s*-triazine. Standards of both compounds were provided. The CIPAC method 91/1/M/-[2] for atrazine was recommended; however, all participants were free to use their own methods. Full particulars of any alternative method had to be submitted with the results. The suspension concentrate was specially prepared in a formulation laboratory and contained approximately 25% atrazine and 6% simazine, which differs from concentrations found in regular formulations. Nine laboratories with ten analysts indicated that they would like to participate and received samples and standards.

ICE 2/82

This exercise was divided into two parts. A sample solution containing methidathion with parathion as the unknown was supplied. The concentration of both constituents and the identity of the unknown had to be determined. A spiking solution also containing the same two pesticides and a sample of frozen orange pulp was also supplied. The concentrations of the two pesticides in the two solutions were not the same. After defrosting the pulp the spiking solution had to be added to it and the mixture used for analysis. Participants were instructed to extract the orange pulp with hexane, to filter the extract and dry it over anhydrous sodium sulphate. This extract could then be analysed without further clean-up or concentration. Each laboratory could use the column and detector of its own choice but had to report the details. The methidathion and parathion concentrations were approximately 51 and 10 mg l^{-1} and 10 and 2 mg kg^{-1} in the solution and pulp, respectively. Ten analysts representing nine laboratories participated in this exercise.

ICE 3/82

Two peanut butter samples were supplied. One was fortified with pirimiphos-methyl and diazinon as the unknown at 8.72 and 5.12 mg kg^{-1}, respectively. The second sample was not fortified and could be used by the participants to determine the recovery of their extraction methods. The method of Sawyer[3] was recommended but all participants were allowed to use the column and detector of their choice, but reporting the details. Results had to be reported on the whole peanut butter sample and not on the oil content. Seven laboratories indicated their willingness to participate and were supplied with samples and standards.

ICE 1/83

A sample of an emulsifiable concentrate containing cypermethrin was supplied to participants. This formulation was specially prepared and contained approximately 170 g kg^{-1} of cypermethrin isomers. Standard solutions of cypermethrin and of an internal standard, tetramethrin, were also supplied. The confidential method of Form-Chem (Pty) Ltd. for the analysis was given to participants and they were requested to follow this method, which utilises a flame-ionisation detector in the GC analysis. Ten laboratories participated in this ICE.

ICE 2/83

A mixture of an organochlorine pesticide, α-endosulfan, and an organophosphate, chlorpyrifos, as the unknown was used in this exercise. The exercise was divided into two parts: a solution containing the two pesticides was supplied and the participants had to quantitate both with standards supplied and then determine the identity of the unknown.

A spiking solution containing the same two pesticides was supplied and had to be mixed with the cabbage pulp (also supplied). The concentrations of the pesticides in the two solutions were not the same. The cabbage pulp was supplied frozen and had to be defrosted before use. The mixture of pesticide and pulp had to be extracted with a hexane - ethyl acetate mixture (6 + 4) and the extract filtered and dried over anhydrous sodium sulphate. The extract could then be used for quantitation without further clean-up or concentration. All the analysts could use the column and detector of their choice, reporting the details. A total of 28 analysts from 20 laboratories participated in this exercise, some, however, only in the first part.

ICE 3/83

Preserved cream fortified with dieldrin and *p,p'*-DDT (dichlorodiphenyltrichloroethane) as the unknown was used in this exercise. The method of Pick *et al.*[4] was recommended, omitting the formalin extraction step, for fat extraction and clean-up by size exclusion chromatography. Laboratories could, however, use their own methods but had to supply full details. Results of the analysis had to be reported on a full-cream basis. Nine laboratories indicated that they would participate and were supplied with samples and standards.

Evaluation of the Results

Results from ICE 1/82 and ICE 1/83 were not evaluated statistically except for the determination of the mean standard deviation and relative standard deviation and the relative standard deviation based on the true value. The acceptability of a result of a formulation analysis was decided using the relevant legislative criteria as laid down under Regulation R2561 of 27 November 1981, article 19, framed under the Fertilizer, Farm Feeds and Agricultural Remedies Act 36 of 1947, which reads: "An agricultural remedy shall not be deemed to deviate in its registered active ingredient contents if an analysis of such agricultural remedy indicated that (a) when it nominally contains less than 25 g of the active ingredient concerned per kg or l, it deviates with not more than 15%; (b) when it nominally contains 25 g or more, but less than 100 g of the active ingredient concerned per kg or l, it deviates with not more than 10%; (c) when it nominally contains 100 g or more, but less than 250 g of the active ingredient concerned per kg or l, it deviates with not more than 6%; (d) when it nominally contains 250 g or more, but less than 500 g of the active ingredient concerned per kg or l, it deviates with not more than 5%; or (e) when it nominally contains 500 g or more of the active ingredient concerned per kg or l, it deviates with not more than 2.5%."

In accordance with a previous decision, results of the other ICEs were not evaluated and laboratories were provided with their results, the true value and the % relative error.[1] This presentation is necessary because statistical methods generally used for evaluation of results of ICEs cannot be applied owing to the small number of participants. Within-laboratory variation was not investigated in these ICEs.

Results

Results were not received from all the participating laboratories so that the total number of analyses reported is sometimes less than expected.

ICE 1/82

Results of this exercise are given in Table 1. Only six of the nine laboratories that originally indicated they would participate supplied results. In total, seven analysts supplied results. Most analysts used the CIPAC method except for two that followed an HPLC method. One sample, D2, was dissolved in

Table 1. Results of ICE 1/82—atrazine suspension concentrate

Laboratory code	Pesticide concentration/g kg^{-1}				Legal acceptability	
	Supplied standard		Own standard			
	Atrazine (known)	Simazine (unknown)	Atrazine (known)	Simazine (unknown)	Atrazine	Simazine
D1	272.3	58.0	271.2	49.6	Not acceptable	Acceptable
D2*	270.0	50.0			Not acceptable	Not acceptable
J	318.0	61.9†	317.9		Not acceptable	Acceptable
L	331.6	Not detected			Not acceptable	
M*	257.0	45.0	258.2	60.3	Acceptable	Not acceptable
Q	251.0	61.0	261.0	49.0	Acceptable	Acceptable
V	261.2	64.1	290.5		Acceptable	Acceptable
Mean	280.2	56.7			Not acceptable	Acceptable
Standard deviation	31.6	7.5				
Relative standard deviation, % of true value	12%	12%				
True value	254.0	61.0				

* An HPLC method was used.
† Simazine was not identified.

Table 2. Results of ICE 2/82—parathion and methidathion solution

Laboratory code	Pesticide concentration: Parathion (unknown) Concentration/mg kg^{-1}	Parathion (unknown) Relative error, %	Methidathion (known) Concentration/mg kg^{-1}	Methidathion (known) Relative error, %	Column used	Detector used*	Deviation between own standard and supplied standard,† %: Parathion	Methidathion
A	47.21‡	—	78.04	53	OV17 + QF1	NPD	89.0	Not carried out
B1	7.12	30	43.24	15	OV17 + QF1	NPD, TLC	5.9	3.2
B2	6.98	31	43.05	16	OV17 + QF1	NPD, TLC	0.3	6.8
C	10.23	1	54.45	7	OV17 + OV210	NPD	4.2	8.4
L	9.86	3	61.60	21	OV101	FPD	51.0	118.0
M	11.50	14	61.40	20	No details	NPD, MS	7.0	5.0
P	19.00	88	49.00	4	OV101	NPD	Not carried out	
T	9.98	12	55.15	8	SE30	FPD	Not carried out	
Mean	10.67		55.74					
Standard deviation	4.04		11.52					
Relative standard deviation, % of true value	40%		23%					
True value	10.13		51.03					

* NPD, nitrogen - phosphorus detector; FPD, flame photometric detector; TLC, thin-layer chromatography for confirmation; and MS, mass spectrometry for confirmation.
† A supplied standard was used for quantitation.
‡ Outlier.

Table 3. Results of ICE 2/82—orange pulp

Laboratory code	Pesticide concentration: Parathion (unknown) Concentration/mg kg^{-1}	Parathion (unknown) Relative error, %	Methidathion (known) Concentration/mg kg^{-1}	Methidathion (known) Relative error, %	Column used	Detector used
A	5.21*	—	9.34	8	OV17 + QF1	NPD
B1	2.24	11	16.14	58	OV17 + QF1	NPD, TLC
B2	2.30	14	15.60	53	OV17 + QF1	NPD, TLC
C†	2.10	4	10.00	2	OV17 + OV210	NPD
L	1.91	6	9.80	4	OV101	FPD
M	1.58	22	7.90	23	No details	NPD, MS
P	2.00	1	3.00	70	OV101	NPD
T	2.28	13	11.21	10	SE30	FPD
Mean	2.06		10.37			
Standard deviation	0.26		4.20			
Relative standard deviation, % of true value	13%		41%			
True value	2.02		10.20			

* Outlier.
† Acetone was used as a solvent.

methanol and an internal standard, 2,4,5-T, was used at the same concentration level as the unknown. The HPLC column was LiChrosorb C-18 with a length of 200 mm and an i.d. of 4.6 mm. A water - methanol gradient was used starting with 68% methanol and changing to 43% after 1.3 min and back to 68% after 7.0 min. A 10-µl volume of sample was injected automatically and the peaks were integrated using base line - base line correction. Unfortunately, the other laboratory, M, gave no details of the HPLC method used.

Four analysts could not determine the atrazine concentration within the legal limits. One of these laboratories used an HPLC method. Only six analysts could determine the simazine but two laboratories had problems with the quantitative analysis.

The mean of the results was not acceptable for atrazine, which means that as a group, the analysts could not determine the concentration within the legal limits. This has serious implications because these analysts are responsible for quality control or are involved in Government laboratories that control marketed formulations.

The comparison of the laboratory standard with the supplied standard indicated that greater care should be taken in ensuring the integrity and purity of standards. In some instances the laboratory standard seemed to have a higher concentration of the pure compound than the supplied standard, while in other instances the situation was reversed.

ICE 2/82

Results of this exercise are given in Tables 2 and 3. Seven of the original nine laboratories returned results. In total, eight analysts were involved. All analysts used the methods given except C who used acetone to extract the orange pulp, then evaporated the acetone and partitioned the aqueous extract into hexane.

The results of the analysis of the solution showed that a number of laboratories experience problems analysing even a simple solution of pesticides. Only dilution and gas chromatography were involved, but nevertheless half the analysts presented results with a percentage relative error in excess of 20% for parathion and three of the eight in excess of 20% for methidathion. The one laboratory, A, whose results were

Table 4. Results of ICE 3/82—peanut butter

Laboratory code	Pesticide concentration: Pirimiphos-methyl (known) Concentration/ mg kg^{-1}	Pirimiphos-methyl (known) Relative error, %	Diazinon (unknown) Concentration/ mg kg^{-1}	Diazinon (unknown) Relative error, %	Column used	Detector used
A	5.49	37	4.02*	22	OV17 + QF1	NPD
M	8.31	5	3.99	22	SE30 + SP2401	NPD - MS
O	8.98	3	52.78†		OV17 + QF1	NPD
T	9.12	5	5.20	2	SP2100	NPD
U	4.77	45	1.46	72	Reoplex + OV101	FPD
Mean	7.33		3.67			
Standard deviation	2.05		1.56			
Relative standard deviation, % of true value	24%		30%			
True value	8.72		5.12			

* Not identified.
† Outlier.

Table 5. Results of ICE 1/83—cypermethrin emulsifiable concentrate

Laboratory code	Cypermethrin concentration/ g kg^{-1}	Legal acceptability	Column used*
C	171.0	Acceptable	OV101
E	171.3	Acceptable	OV101
F	195.6	Not acceptable	SE30
G1	169.5	Acceptable	OV101
G2	167.0	Acceptable	OV101
J	197.8	Not acceptable	OV101
N†	184.5	Not acceptable	OV101
P‡	173.2	Acceptable	OV17
R	173.0	Acceptable	OV101
U	168.8	Acceptable	OV101
Mean	177.2		
Standard deviation	11.3		
Relative standard deviation, % of true value	7%		
True value	172.0	±10.3	

* All laboratories used a flame-ionisation detector.
† An external standard was used.
‡ Leopynamin was used as an internal standard.

considered outliers did not dilute the solution provided and probably worked outside the linear region of the detector.

Analysis of the orange pulp gave results again deviating far from the true value. Half of the analysts gave answers for the known compound that were more than 20% higher than the true value. The results for parathion were closer to the true value although two of the laboratories reported results more than 20% greater than the true value.

ICE 3/82

Results for this exercise are presented in Table 4; only five of the original seven laboratories eventually provided results. The known pesticide, pirimiphos-methyl, which is often used for treatment of storage facilities of grain, was accurately determined by three laboratories. The unknown compound, diazinon, was not identified by one laboratory. Four of the five participants reported results within 10% of the true value and the outlier was obviously the result of a calculation error. Only one laboratory confirmed the identity of the unknown with a different technique, which was mass spectrometry. The other laboratories relied on the relative retention index, making use of only one column.

ICE 1/83

Results of this formulation analysis are reported in Table 5. Nine of the analysts who indicated they would participate supplied results but one additional analyst joined the exercise. The strict legal limit of a maximum 6% deviation was enforced, which meant that the results of three analysts were not acceptable. All three analysts made the same mistake of not correcting for the purity of the standard, given as 90%. This was surprising because all these analysts were experienced and should have applied a correction factor.

ICE 2/83

Results of this exercise are given in Tables 6 and 7. Only 13 of the original 20 laboratories and 15 out of the original 28 analysts eventually supplied results. The analysis of the solution of two pesticides was carried out for the known compound endosulfan to within 20% by nine of the analysts and for the unknown chlorpyrifos to within 20% by 11 of the analysts. Two laboratories produced results that were outliers for both compounds, notwithstanding the use of standards supplied and following the method as given. Most laboratories identified the unknown correctly as chlorpyrifos using relative retention indices and a selective detector such as a nitrogen - phosphorus detector. Four laboratories also used a mass spectrometer to identify chlorpyrifos.

The determination of the concentration of the two pesticides in the cabbage pulp was carried out to within 20% of the true value by only six laboratories for α-endosulfan and by five laboratories for chlorpyrifos. This means that less than 50% of the analysts could produce a result that was within 20% of the true value.

ICE 3/83

Results are given in Table 8. Six of the nine laboratories supplied with samples eventually reported their results. Three laboratories gave the results of two analysts. The results were in most instances close to the true value; however, none of the laboratories confirmed the identity of the unknown compound *p,p'*-DDT. This is surprising because most of the participants had access to mass spectrometers or other techniques for confirmation. The one laboratory using a capillary column could determine the *p,p'*-DDT concentration accurately but not that of the dieldrin.

Discussion

The results of the ICEs carried out with pesticide formulations showed that a large number of the formulation analysts could

Table 6. Results of ICE 2/83—endosulfan and chlorpyrifos solution

Laboratory code	Pesticide concentration: α-Endosulfan (known) Concentration/ mg kg^{-1}	α-Endosulfan (known) Relative error, %	Chlorpyrifos (unknown) Concentration/ mg kg^{-1}	Chlorpyrifos (unknown) Relative error, %	Column used	Detector used	Deviation between own standard and supplied standard, % α-Endosulfan	Deviation between own standard and supplied standard, % Chlorpyrifos
B	10.30	1	5.10	2	SP2100	ECD - FPD	3.5	0
C	9.93	3	4.92	5	SE30 + QF1	ECD	*	*
D	10.13	1	5.16	1	OV101	ECD	3.0	0.4
G†	9.80	4	5.70	10	OV101‡	ECD - NPD - MS	40.9	1.2
I	10.31	1	4.49	13	OV101 + OV210	ECD - MS		
K	11.10	9	4.40§	15	OV101	ECD - NPD	*	*
L	9.92	3	3.58¶	31	OV1‡	ECD	*	*
M1	15.21	49	5.13	1	OV17 + QF1	ECD - NPD - MS	0	7.5
M2†	13.10	29	5.25	1	OV17 + SP2401	ECD - NPD	3.0	1.3
O	43.90‖	—	18.7§,‖	—	SE30	ECD	*	*
Q	43.20‖	—	18.4§,‖	—	OV17 + OV210	ECD	*	*
N	9.75	4	5.25	1	OV101	ECD	*	*
S1	16.71	64	9.29§	79	OV17 + QF1	ECD	*	*
S2	19.81	94	5.38§	4	OV17 + QF1	ECD - NPD	2.0	*
W	10.45	3	4.68	10	SE30 + QF1	ECD - MS		
Mean	12.04		5.26					
Standard deviation	3.24		1.33					
Relative standard deviation, % of true value	32%	26%						
True value	10.19		5.19					

* Not carried out.
† An internal standard was used.
‡ A capillary column was used.
§ Not identified.
‖ Outlier.
¶ Identified as chlorofenphos.

Table 7. Results of ICE 2/83—cabbage pulp

Laboratory code	Pesticide concentration: α-Endosulfan (known) Concentration/ mg kg^{-1}	α-Endosulfan (known) Relative error, %	Chlorpyrifos (unknown) Concentration/ mg kg^{-1}	Chlorpyrifos (unknown) Relative error, %	Column used	Detector used
B	2.52	38	1.24	40	SP2100	ECD - FPD
C	3.93	4	2.13	3	SE30 + QF1	ECD
D	4.20	3	2.20	6	OV101	ECD
G*	5.55	36	3.70	79	OV101†	ECD - NPD - MS
I	2.03	50	1.35	35	OV101 + OV210	ECD - MS
K	17.30‡	—	3.40§	64	OV101	ECD - NPD
M1	3.02	26	1.23	41	SE30 + SP2401	ECD - NPD - MS
M2*	3.63	11	1.70	18	SE30 + SP2401	ECD - NPD
Q	39.60‡	—	15.40‡	—	OV17 + OV210	ECD
S1	3.64	11	1.77	17	OV17 + QF1	ECD - NPD
S2	4.04	1	2.70	30	DC200	ECD - NPD
W	4.50	10	2.15	4	OV101 + OV210	ECD - MS
Mean	3.71		2.14			
Standard deviation	1.01		0.84			
Relative standard deviation, % of true value	25%		41%			
True value	4.08		2.07			

* An internal standard was used.
† A capillary column was used.
‡ Outlier.
§ Not identified.

not determine the concentrations correctly, *i.e.*, not within the legal limits. This may have far-reaching implications because not only can the analysts not provide good quality control in the factory but they may also complicate control measures by not ensuring that the farmer receives an up-to-standard pesticide. This may lead to serious problems in farming and may cause the appearance of resistant pests and may also give the pesticide an undeservedly bad reputation. Fortunately, the feedback experienced by the Task Group after each exercise showed that these analysts take their mistakes seriously. They actually clamored for more ICEs dealing with formulations and a separate Task Group on ICE (Formula-

Table 8. Results of ICE 3/83—cream

Laboratory code	Pesticide concentration: Dieldrin (known) Concentration/ mg kg^{-1}	Dieldrin (known) Relative error, %	*p,p'*-DDT (unknown) Concentration/ mg kg^{-1}	*p,p'*-DDT (unknown) Relative error, %	Method used	Column used
C	0.058	7	0.110	8	As given	OV17 + QF
D	0.035	26	0.085	12	As given	OV101 + OV210
I1	0.040	26	0.060	41	As given	OV101 + OV210
I2	0.030	44	0.070	31	Alumina clean-up	OV101 + OV210
L	0.128*	—	0.069	31	HPLC clean-up	Phenyl methyl silicone†
M1	0.024	56	0.067	34	As given	OV17 + QF1
M2	0.023	57	0.057	44	As given	OV17 + QF1
S1	0.060	11	0.080	22	Florosil clean-up	OV17 + QF1
S2	0.060	11	0.080	22	Florosil clean-up	OV17 + QF1
Mean	0.04		0.075			
Standard deviation	0.016		0.016			
Relative standard deviation, % of true value	30%		16%			
True value	0.054		0.102			

* Outlier.
† A capillary column was used.

tions) was founded to cater for their demands. This experience shows the value of ICEs and should serve as a strong stimulus for the members of the Task Group who often feel discouraged by the low returns of completed analyses.

The results of the residue analysis ICEs were also not very encouraging. Surprisingly, the most simple type of analysis of a solution containing two pesticides was carried out badly by many analysts. Only gas chromatography and dilution were tested but even so, many laboratories reported outliers. This indicates a basic need for the training of analysts adept at the most fundamental analytical techniques. A large number of laboratories analyse fatty and non-fatty vegetable matrices daily but even these laboratories still do not perform very well.

Most laboratories in South Africa are well equipped with modern instruments and most have access to sophisticated techniques such as mass spectrometry. Nevertheless, it seems that most are still contented to identify pesticides by making use of retention indices only and sometimes doing this on only one column. This indicates the need for educating analysts properly in the pitfalls of pesticide analysis. The Working Group on Pesticide Analysis regularly organises symposia to help the analysts to understand the difficulties in pesticide analysis and how to overcome them. The Task Group noticed with interest the increased use of capillary gas chromatography and the use of HPLC in formulation analysis. These techniques are of great use to pesticide analysts and their use should be encouraged.

Appendix: Participating Laboratories

Laboratories of the following organisations indicated their willingness to participate in ICEs: Agbro (Pty) Ltd.; Agricultural Product Standards Division, Department of Agriculture; Agrochem (Pty) Ltd.; Ciba-Geigy SA (Pty) Ltd.; City Health Department, Johannesburg City Council; Coopers RS (Pty) Ltd.; Delta G (Pty) Ltd.; Echalaz and Osborne (Pty) Ltd.; Food and Nutritional Products SA (Pty) Ltd.; Form-Chem (Pty) Ltd. (two laboratories); Health Chemistry Laboratory, Department of Health and Welfare; Oil Seeds Board; Maize Board; Marine Pollution Group, University of Port Elizabeth; Plant Protection Research Institute, Department of Agriculture; Shell Chemicals SA (Pty) Ltd.; Soil and Irrigation Research Institute, Department of Agriculture; South African Bureau of Standards (two laboratories); South African Transport Service; Union Carbide SA (Pty) Ltd.; Vetsak (Pty) Ltd.; and Water Research Institute, Council for Scientific and Industrial Research.

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A Computer Search System for the Identification of Drugs Using a Combination of Thin-layer Chromatographic, Gas - Liquid Chromatographic and Ultraviolet Spectroscopic Data

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A computer-based retrieval system has been developed for the identification of drugs and poisons in forensic toxicology using thin-layer chromatographic, gas - liquid chromatographic and ultraviolet spectroscopic data. The data collection has been compiled from various published and unpublished sources and contains information for over 1600 compounds. The retrieval program uses the concept of discrepancy index to match experimental data for an unknown with the information in the data file. Additional features include a series of help routines for new users and the facility to retrieve information on specific compounds or groups of compounds in the data collection.

Keywords: *Drug identification; computer retrieval; thin-layer chromatography; gas - liquid chromatography; ultraviolet spectroscopy*

A toxicological investigation in forensic science to identify the presence of an unknown drug or poison in pharmaceutical preparations or body fluids is generally a stepwise process involving a wide range of analytical techniques. The early stages in this process require techniques that are capable of detecting a wide range of compounds as rapidly as possible. In addition, these screening techniques should be sufficiently robust for routine use and, ideally, inexpensive. Ultraviolet (UV) spectroscopy has been widely used for drug screening procedures, but can be subject to interferences from substances co-extracted with the compounds of interest. For this reason, separation techniques such as thin-layer chromatography (TLC) and gas - liquid chromatography (GLC) are widely used. In recent years, immunoassay methods have also been applied to the detection of particular drug classes in body fluids and tissues.[1] Inevitably, such screening methods rarely give a unique identification of an unknown compound and the preliminary findings need to be confirmed with more discriminatory techniques such as infrared (IR) spectroscopy and mass spectrometry. Once the identity of a potentially toxic substance has been firmly established, it can be quantified and this analytical result can then be interpreted.

The use of chromatographic techniques for drug identification involves the comparison of the retention properties of the unknown with those of reference compounds. Considerable effort has been directed towards the selection of optimum chromatographic systems and the combination of these systems for drug identification.[2-11] The effectiveness of a chromatographic system for identification purposes is related to the number of compounds that may be encountered at each unique position across the chromatographic range. The number of unique positions is controlled by the chromatographic resolution (*e.g.*, GLC resolves more compounds than TLC), whereas the number of compounds to be found at each position is determined by the frequency distribution of the data for all reference compounds. Using these ideas, embodied in the concept of discriminating power, a wide range of systems has been evaluated, leading to the selection of eight TLC systems (four for basic drugs and four for acidic and neutral drugs)[12] and a GLC system[13] for routine drug screening. These systems show good discrimination when used individually, but this can be further improved by using them in combination. Further, inter-laboratory trials within UK Forensic Science Laboratories have shown them to be robust with relatively low coefficients of variation of the retention measurements.

Less progress has been made towards the standardisation of recording UV spectra in drug screening procedures. It is common practice to measure the UV spectrum of an unknown compound under different pH conditions as shifts in UV absorption maxima can provide valuable information.[14] However, a wide range of different solvents have been used, including water, methanol, ethanol, aqueous and alcoholic hydrochloric and sulphuric acid, aqueous borax buffer and aqueous sodium hydroxide. In addition, the concentrations of acids and alkalis have varied considerably. Nevertheless, apart from the effect of pH, the solvent selected has a relatively small influence on the UV spectrum. Inter-laboratory trials involving 11 different spectrophotometers have indicated that the reproducibility of measuring the positions of absorption maxima is within 2 nm.[6]

After the selection of the appropriate analytical systems, the next stage in the standardisation of drug screening procedures has involved the generation of libraries of reference data for compounds likely to be encountered in forensic casework. UV absorption data are available in standard texts[15] and other unpublished sources[16] and TLC data for over 700 compounds and GLC data for over 1300 compounds, all measured on the recommended systems discussed above, have been published.[12,13] These sources provide a valuable data base for those involved with drug identification, but the presentation of the information in printed tables has severe limitations. The printed data quickly become out of date as new compounds are introduced and new information is generated for existing compounds. However, the most serious problem is that searching tabular data is very time consuming and often difficult. Further, for a large data base it is essential that a set of tables is available with each containing the same information, but presented in different ways to simplify searching. Firstly, it is always desirable to have a table with the data arranged in alphabetical order of the compound names to facilitate the rapid retrieval of information for any compound when the presence of that compound is suspected from case details. It is then necessary to have further tables (one for each system) with the compounds arranged in the numerical order of the data (*e.g.*, order of elution). Thus, by setting an appropriate error window around the value measured for the unknown compound, it is possible to arrive at a list of possible identities. This process can be repeated for each system for

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which experimental data are available. At the end of the search it is hoped that these lists overlap and indicate the true identity.

The use of a computerised data base with an appropriate retrieval program overcomes the major problems associated with the use of printed tables. Further, it is possible to introduce a more rigorous approach to comparing the experimental data with the reference values and thus replace the subjective methods often applied to searching tabular data. So far little work has been published on computerised search systems for chromatographic retention data. Musumarra *et al.*[17] applied principal components analysis to the identification of unknown drugs after retention measurement on four TLC systems using a data base for 596 drugs taken from our published review.[12] In capillary gas chromatography Newton and Foery[18] described a computerised window search system for drug identification in emergency toxicology, while a commercial "Retention Index Library" has been announced[19] that also uses a window search and allows cross-correlation of search results from two different columns. The use of a computerised search system that allows the combination of data from different types of chromatographic system has been described by Schepers *et al.*[20] using the concept of "mean list length."

It is surprising that computerised search systems for chromatographic retention data have not been widely developed because, in principle, it is relatively straightforward as each compound is represented by a single number on each system. In contrast, much interest has been directed towards the use of computers for the storage and retrieval of spectroscopic data (*e.g.*, mass spectrometry and IR spectroscopy). Such data are two-dimensional (*e.g.*, ion intensity *vs.* *m/z* in mass spectrometry), making the problem of comparing spectra much more complex. A wide range of algorithms have been applied but, generally, they all involve an initial simplification of the two-dimensional data array (*e.g.*, selecting the eight most intense peaks in a mass spectrum, selecting the positions of the peak maxima in an IR spectrum), followed by the calculation of a defined parameter that quantifies the similarity between the spectra. This approach allows the possible identities to be listed in rank order. Recent reviews[21,22] on the historical development of retrieval systems in mass spectrometry illustrate well the general principles involved in searching spectroscopic data and the approaches that have been adopted to improve both speed and accuracy. Recent work involving gas chromatography in combination with mass spectrometry has demonstrated that the inclusion of retention data in the search system can greatly improve the reliability of matching.[23]

This paper describes a computerised data base of GLC, TLC and UV spectroscopic information and the development of a suitable search program to aid the forensic scientist in the screening of unknown drugs and poisons in forensic science.

Data Base

The data base was set up on a Prime 550 computer situated at the Central Research Establishment, Home Office Forensic Science Service (Aldermaston). This computer is linked by telephone lines to ten other Forensic Science Laboratories throughout the UK with access through about 60 computer terminals each having a keyboard and a visual display unit (VDU). Facilities for obtaining printed reports are available in all laboratories.

The computer data file is arranged alphabetically and, to allow effective retrieval of information, all compounds were named according to standard texts. The names are those given in Martindale (27th Edition)[24] wherever possible or the Merck Index (9th Edition)[25] or the Nanogen Index (1975)[26] for the economic poisons. The file contains information for 1605 compounds of forensic interest and includes agricultural chemicals, plasticisers and putrefactive substances in addition to acidic, basic and neutral drugs. Only highly reliable data have been used, obtained from a number of published and unpublished sources, *viz.*, TLC[12], GLC[13] and UV spectroscopy.[15,16]

Each compound in the data file is represented by two lines of information when displayed on the VDU screen or when a printout is obtained. Table 1 shows a small section of the file. The first line gives the name of the compound and the second line contains the analytical data. For some compounds a second name in parentheses is included on the first line after the official name, *e.g.*, Lysergide (LSD). The data are arranged in 15 columns, each representing a different analytical system and headed by code letters that are explained in

Table 1. Part of the computer file showing the standard format of data presentation. Column headings are explained in Table 2

A	B	C	D	E	F	G	H	GLC	UVA	UVB	UVC	UVD	UVE	$A^{1\%}_{1\,cm}$
Oestrone														
								2612					287	0080E
Opipramol														
54	06	22	07						255					0734A
Orciprenaline														
48	01	03	06					9999	276					0087A
Orphenadrine														
55	48	33	16					1936	264					0024A
Orthocaine														
79	01	37	50					1655	253					0836A
Oxanamide														
								1249						
Oxazepam														
56	00	40	51	22	44	37	42	2336						
Oxedrine														
25	04	01	18					1705	273			272		0096A
Oxeladin														
50	51	22	19					2198	259					0007A
Oxethazaine														
52	10	07	15					2525	258					0013A
Oxomemazine														
								2725						
Oxprenolol														
48	11	11	13					1870						

Table 2. Types of data contained in the computer file

Code	Description	Data: Units	Data: Acceptable values
A	TLC on silica* (methanol - ammonia, 100 + 1.5)	$R_F \times 100$	0–100
B	TLC on silica* (cyclohexane - toluene - diethylamine, 75 + 15 + 10)	$R_F \times 100$	0–100
C	TLC on silica* (chloroform - methanol, 9 + 1)	$R_F \times 100$	0–100
D	TLC on silica* (acetone)	$R_F \times 100$	0–100
E	TLC on silica (chloroform - acetone, 4 + 1)	$R_F \times 100$	0–100
F	TLC on silica (ethyl acetate - methanol - ammonia, 85 + 10 + 5)	$R_F \times 100$	0–100
G	TLC on silica (ethyl acetate)	$R_F \times 100$	0–100
H	TLC on silica (chloroform - methanol, 9 + 1)	$R_F \times 100$	0–100
GLC	GLC using SE-30 or OV-1	RI	600–4000, 9999†
UVA	UV absorption maximum in acid (pH 1)	nm	230–750
UVB	UV absorption maximum in alkali (pH 13)	nm	230–750
UVC	UV absorption maximum in buffer (pH 10)	nm	230–750
UVD	UV absorption maximum in water	nm	230–750
UVE	UV absorption maximum in ethanol or methanol	nm	230–750
$A^{1\%}_{1\,cm}$	Specific absorbance at UV absorption maximum	Absorbance units	—

* Plates dipped in 0.1 M potassium hydroxide solution and dried.
† Arbitrary value of 9999 assigned to compounds showing retention indices >4000 or not eluting.

Table 2. This table also shows the units used to record the information for each system and the limiting values adopted.

The first eight columns shown in Table 1 are for TLC data with systems A–D and E–H having been recommended for basic and for acidic and neutral drugs, respectively.[12] The TLC data in the file have been corrected by the use of reference compounds[12] and, consequently, it is essential to follow this procedure when analysing unknowns in order to achieve true compatibility with the data base. The next column contains GLC retention data on low-polarity dimethylsilicone stationary phases (SE-30 and OV-1) that have been recommended for drug identification work.[13] Acceptable values for the retention index (RI) are between 600 and 4000, whereas all compounds showing retention indices >4000 or those not eluting have been assigned an arbitrary value of 9999. Some compounds can undergo decomposition in GLC, giving multiple peaks (*e.g.*, chlordiazepoxide), and these are accommodated by multiple entries in the data file, *i.e.*, the compound name appears several times with each entry having a full set of TLC and UV data but a different GLC retention index.

The remaining columns contain the UV spectroscopic data. Space is provided for UV absorption maxima in five different solvents, partially reflecting the lack of standardised procedures in this area. Drugs showing more than one UV absorption maximum can be accommodated by multiple entries in the data file as outlined above for multiple peaks in GLC. No absorption maxima below 230 nm are accepted as this region is subject to problems from scattered light, bacterial metabolites in water and solvent absorption effects. Thus, no entry in the UV columns is possible when a drug shows strong end-absorption at low wavelengths but no absorption maximum. The last column contains the specific absorbance at the UV maximum, $A^{1\%}_{1\,cm}$ (*i.e.*, the absorbance of a 1% *m/V* solution in a 1-cm cell). Whenever a value is included in this final column it is followed by a letter that signifies the solvent in which the result was obtained. In this context the letters A–E in Table 1 correspond to the UV absorption maxima reported in solvents UVA–UVE in Table 2, respectively. For example, oestrone in Table 1 has an $A^{1\%}_{1\,cm}$ of 80 in system UVE, *i.e.*, in ethanol or methanol.

It is important to note that the data collection does not contain a full set of values on all 15 analytical systems for any compound. Values for some compounds have not yet been measured on the appropriate systems, but it is hoped that these deficiencies can be rectified in due course. However, in most instances the chemical nature of a compound has determined which analytical systems are not useful and where the acquisition of data would be inappropriate or impossible. For example, the application of the four acidic and neutral TLC systems to basic drugs would be inappropriate and hence few compounds have data for all eight TLC systems. At present, the data file contains GLC retention indices for approximately 1300 compounds and $R_F \times 100$ values on at least four TLC systems for 794 compounds. For the UV spectroscopic systems (UVA–UVE) data are available for 389, 59, 22, 38 and 133 compounds, respectively.

Principle of the Analytical Data Search System

The underlying principle of the search system to match the analytical data of an unknown against the information contained in the data file is the concept of discrepancy index (*DI*), first introduced by Parker.[27] For the comparison of N analytical parameters i ($i = 1, 2, \ldots, N$), *DI* is defined by

$$DI = \sum_{i=1}^{N} [(X_i - x_i)/\sigma_i]^2$$

where X_i is the reference value for each analytical parameter, x_i is the experimental value and σ_i is the standard deviation observed for the measurement of the reference value.

The *DI* gives a quantitative estimate of the match between a set of experimental values and the reference values for these same analytical systems for each compound in the data base. *DI* is zero when the experimental data and reference data match perfectly and increases as the two sets of data diverge. The *DI* allows for each analytical system to have a different level of precision and in this respect differs from other parameters used to compare analytical data. The values for σ_i in the calculation of *DI* are 2 for the five UV spectroscopic systems (UVA–UVE), 5 for the eight TLC systems (A–H) and 17 for the GLC system. These numbers are based on intra- and inter-laboratory trials to estimate standard deviations[6,12,13] for the analytical systems and the experience gained using the data in tabular form for drug identification.

Provided that the analytical parameters are mutually independent, *DI* has a χ^2 distribution with N degrees of freedom.[27] Thus, for a given number of analytical systems, it is possible to relate the value of *DI* with a certain probability that the data for the unknown compound could have been generated by the particular reference compound. The list of possible matches can then be restricted to those compounds

with DI values less than a critical value ($DI_{max.}$) corresponding to an appropriate statistical significance level. Table 3 shows the relationship between the number of systems and the $DI_{max.}$ value at two significance levels (1 and 5%). The search program described in this paper uses the $DI_{max.}$ values corresponding to the 1% level.

Initial studies to test the concept of DI for the identification of drugs from TLC data have been reported.[28] The work involved a small data collection for 100 basic drugs and a program written for the Hewlett-Packard 2100A computer.

Computer Search Program

The search program, called DISCREP, is one of the options offered on the Prime 550 computer for use by forensic science laboratories in the UK. It is an interactive menu-based program written in FORTRAN that checks all inputs by the user to ensure that the correct type of information is being supplied. A number of help options are included to assist new users. There are two main options (A and B) that allow retrieval based on analytical data or compound names, respectively, and these are described below.

Option A (Searching on Analytical Data)

This option allows the user to compare a set of experimental values for an unknown with the data base and obtain a list of possible matches. The user enters the code for an analytical system followed by the experimental value for that system. This process is repeated until the complete set of data have been entered and the search is started. Some reference compounds will have data corresponding to all the analytical systems entered for the unknown, while others will only have data for some of the systems and hence the number of systems compared (N) will change from compound to compound. The program calculates DI for the experimental data in combination with each reference compound and compares this value with $DI_{max.}$ whose value is determined by the appropriate value of N. Those compounds with $DI < DI_{max.}$ are described as hits and can be displayed as likely matches.

The results are displayed in a form similar to that in Table 1 except that the data line includes the DI value followed by the number of analytical systems for which data have been compared. A typical display is shown in Table 4. The data are displayed in blocks, each corresponding to compounds where the same number of systems have been compared. Within each block the hits are listed in order of increasing DI, *i.e.*, decreasing likelihood of matching. When several compounds have the same DI value they are listed in alphabetical order. It is important to remember this block structure when using the program and not to treat the display of hits as a continuous list with decreasing likelihood of match. The true match may appear in a lower block and this only indicates that data have been obtained for the compound (as an unknown) where such information is not included in the data base. It is also important to remember that DI is not a measure of how close the experimental data fit the reference data, but is actually a measure of the likelihood of the experimental data being generated by the reference compound. When the list of hits includes several likely matches with similar DI values, the full set of data available for these compounds enables the user to select which of the remaining analytical systems, not included in the original search, will give maximum discrimination. The analyst can then perform the next stage of the analysis with a high probability of obtaining a clear identification.

All analytical systems (Table 2) can be searched using the

Table 3. Critical values for the discrepancy index ($DI_{max.}$) at two significance levels

Number of analytical systems (N)	Values for $DI_{max.}$ 5% level	1% level
1	3.8	6.6
2	6.0	9.2
3	7.8	11.3
4	9.5	13.3
5	11.1	15.1
6	12.6	16.8

Table 4. Part of the results from a search using three analytical parameters*

A	B	C	D	E	F	G	H	GLC	UVA	UVB	UVC	UVD	UVE	$A^{1\%}_{1cm}$	DI	N
Unknown data supplied																
		62						1552	265							3
Leptazol																
72	07	64	63					1552	265					0004A	0.15	3
Nikethamide																
59	15	56	29					1525	264	260				0273A	4.21	3
Etenzamide																
64	03	59	55					1542							0.70	2
Phenprobamate																
				47	74	55	60	1520	262					0012A	5.79	2
Pyridostigmine bromide																
								1515	269			269		0180A	8.73	2
3,4-Dimethoxyphenethylamine																
								1551							0.00	1
Ergotoxine																
66	01	62	48												0.00	1
Acetazolamide																
				04	03	31	18		265					0474A	0.00	1
Tributyrin																
								1552							0.00	1
Crotamiton																
								1550							0.01	1
Clofibrate																
				75	82	66	71	1549							0.03	1
Ethylallylbarbitone																
								1555							0.03	1
Thurfyl nicotinate																
60	31	63	50												0.03	1

* See Table 2 for details of the analytical systems.

present program, with the exception of the specific absorbance values ($A^{1\%}_{1\,cm}$). In addition, compounds assigned an arbitrary retention index of 9999 in the data file cannot be searched using this value. When compounds give more than one experimental value for a particular analytical system (*e.g.*, multiple peaks in GLC arising from thermal decomposition, several absorption maxima in the UV spectrum) it is advisable to repeat the search procedure using each of the experimental values in turn. This ensures that a drug is not overlooked as a possible match if the data file does not contain all the experimental values.

At this stage in the search program the user can file the results for later examination or use data from a further analytical system and perform another search. This latter feature is particularly useful when there is uncertainty over a particular value in a set of experimental measurements when the influence of including this extra information in a second search can be observed.

Option B (Searching on Compound Names)

There are two types of search available under this option. The first sub-option allows the retrieval of data for a specific compound. The user types in the full name (including all commas, parentheses and hyphens) and the analytical data for the compound are displayed in a manner similar to that shown in Table 1. This feature can be particularly useful when a search on analytical data for an unknown (option A) does not indicate a match for a particular compound mentioned in case details. The data for this compound can then be retrieved and examined for closeness of fit with the experimental data.

The use of the first sub-option can be misleading when the file entry for the required compound contains alternative names. For example, a search for acetylsalicylic acid gives no information because the entry in the data file is "Acetylsalicylic Acid (Aspirin)." The second sub-option solves this problem by allowing the user to retrieve all compounds whose names include a specified name fragment. Hence a search using either "Acetylsalicylic Acid" or "Aspirin" can be used to retrieve the data required. This sub-option can also be used for the retrieval of data for drug metabolites when the exact names or the positions of hyphens, commas, etc., are not known. For example, a user requiring data on a hydroxylated desalkyl flurazepam metabolite can type in "FLURAZ," giving data for *N*-1-desalkyl-3-hydroxyflurazepam together with those for other compounds containing this name fragment. A similar principle can be applied when searching for quaternary ammonium compounds where the nature of the anion is unknown, *e.g.*, emepronium bromide. A further use of this sub-option involves the retrieval of information for groups of compounds within a particular drug class. For example, the names of most barbiturates include the fragment "BARB" (*e.g.*, phenobarbitone, amylobarbitone) and the names of many benzodiazepines include the fragment "PAM" (*e.g.*, diazepam, flurazepam). Clearly, the effectiveness of such searches depends on the consistency of the naming within the class. Thus the barbiturate talbutal and the benzodiazepine triazolam are not retrieved in the examples above. Nevertheless, the use of this approach can be particularly useful for comparing the properties of compounds within a drug class and selecting appropriate analytical systems to give maximum discrimination.

Evaluation of the Search Program

The use of the search program for finding correct matches for experimental data from "unknown" compounds has been evaluated using nine basic drugs (Table 5). The drugs were selected as being representative of the major drug classes (*viz.*, central stimulants, phenothiazines, local anaesthetics, opiates, antidepressants, benzodiazepines, antihistamines and analgesics/anti-inflammatories), and in each instance the data file contained values for the TLC systems (A–D), GLC and UVA. Table 5 shows the reference values for the compounds and the values that have been used to search the data base are given in parentheses. Apart from the UV absorption maximum of benzydamine (308 nm), none of the values were unusual or offered more than average discrimination in any system. The search data were not obtained experimentally, but by the addition of 1.645 times the inter-laboratory standard deviation[12,13] to the TLC and GLC reference values representing the upper limits for 90% of all expected values. The search values for UV spectroscopy involved the addition of 1 nm to the reference values.

The results of the evaluation are shown in Table 6, where various combinations of the analytical data have been used. For each of the searches the position of the true match in the list of hits over the total number of hits is given. For example, the result for nikethamide with a search involving TLC data on four systems (ABCD) indicates that the compound was located at the top (lowest *DI* value) of a list of 15 hits (1/15). This presentation is only misleading when two or more compounds give identical *DI* values when the program lists these in alphabetical order. This is a particular problem with searches involving UV spectroscopy alone, *e.g.*, nitrazepam is shown as 17th out of 74 hits (Table 6), but is really joint 2nd.

The first part of Table 6 contains the results of searches to investigate the effects of combining data from the three types of analytical technique (TLC, GLC and UV). For these searches the four TLC systems (ABCD) have been treated as

Table 5. Analytical data for nine compounds on six analytical systems used in the evaluation of the search system*

Drug	Reference and search data†					
	A	B	C	D	GLC	UVA
Nikethamide	59 (63)	15 (19)	56 (62)	29 (34)	1525 (1552)	264 (265)
Lignocaine	70 (74)	35 (39)	73 (79)	63 (68)	1870 (1897)	263 (264)
Promazine	44 (48)	41 (45)	30 (36)	11 (16)	2316 (2343)	251 (252)
Hydrocodone	25 (29)	4 (8)	20 (26)	4 (9)	2440 (2467)	280 (281)
Nitrazepam	68 (72)	0 (4)	36 (42)	55 (60)	2750 (2777)	280 (281)
Iproniazid	69 (73)	1 (5)	23 (29)	17 (22)	1593 (1620)	267 (268)
Carbinoxamine	48 (52)	25 (29)	19 (25)	4 (9)	2080 (2107)	264 (265)
Chlorpheniramine	45 (49)	33 (37)	18 (24)	2 (7)	2002 (2029)	265 (266)
Benzydamine	44 (48)	36 (40)	22 (28)	9 (14)	2368 (2395)	308 (309)‡

† Reference value (search value). Each search value (TLC and GLC) exceeds the reference value by 1.645 × inter-laboratory standard deviation for the particular analytical system[12,13] and thus represents an upper limit for 90% of all expected values. The search value for UV spectroscopy is the reference value + 1 nm.

* See Table 2 for details of the analytical systems.

‡ Unusual value in this analytical system.

Table 6. Results of searches for nine basic drugs using data on up to six analytical systems*

System(s)†	Relative position‡									
	Niketh-amide	Ligno-caine	Promazine	Hydro-codone	Nitraze-pam	Iproniazid	Carbinox-amine	Chlor-pheniramine	Benzyd-amine	Mean§
ABCD	1/15	10/33	22/68	1/15	5/31	2/22	6/57	14/55	21/72	9/41
GLC	30/49	44/75	43/79	52/73	18/29	35/58	42/70	55/76	48/73	41/65
UVA	19/74¶	19/82¶	12/80¶	11/74¶	17/74¶	13/70¶	21/74¶	11/73¶	6/10¶	14/68
ABCD + GLC	1/70	1/94	3/71	1/70	1/35	1/77	2/77	2/83	2/72	2/72
ABCD + UVA	1/41	1/52	3/54	1/24	1/33	1/38	1/61	1/55	1/28	1/43
GLC + UVA	2/64	3/92	2/71	5/92	1/44	1/67	2/77	4/84	1/68	2/73
ABCD + GLC + UVA	1/74	1/98	1/68	1/74	1/33	1/78	1/87	1/82	1/69	1/74
C + GLC	4/87	2/91	7/101	4/102	2/61	5/93	6/106	7/105	5/98	5/94
AB + GLC	1/84	2/90	4/71	3/83	1/69	2/101	2/80	2/80	1/72	2/81
ABC + GLC	1/71	1/90	3/69	1/69	1/48	1/77	2/74	3/75	2/64	2/71
C + GLC + UVA	2/80	1/94	1/89	1/94	1/49	1/90	2/96	1/97	1/79	1/85

* Search data from Table 5.
† See Table 2 for details of the analytical systems.
‡ Position in list of hits/number of hits.
§ Mean values for all nine drugs rounded to nearest whole numbers.
¶ Equal 2nd place in the list of hits.

a set and were always used in combination. It can be seen that neither TLC nor GLC showed outstanding discrimination when used alone and the correct matches were often low down in the list of hits. In contrast, UV spectroscopy gave a high list position for all the drugs (equal 2nd), but this would not greatly facilitate the identification of an unknown because of the large number of hits with identical absorption maxima. The use of two different techniques (*i.e.*, ABCD + GLC, ABCD + UVA or GLC + UVA) gave dramatic improvements in the success of the retrieval, although there was little to choose between the three possible combinations. As expected, the combination of all three techniques (ABCD + GLC + UVA) gave the best results and the correct match appeared at the top of the list of hits for all nine drugs.

In practice, it could prove to be very time consuming to adopt some of the successful combinations of systems considered above because of the time required to measure the appropriate analytical values. It is interesting to compare these results with a more pragmatic approach that aims to obtain maximum information with the minimum effort. The GLC system shows the largest discrimination for a single chromatographic system,[6] while the amount of retention data for this system exceeds any other at present. Consequently, GLC should figure prominently in any toxicological screening procedure. Once GLC has been used, the combination with one, two or three TLC systems should be considered. The TLC systems have been selected as those combinations which give the best discriminating powers. System C has the highest discriminating power of the four TLC systems when used alone while the combinations A + B and A + B + C give the highest values when two or three systems are run simultaneously.[12] Table 6 shows that the combination of GLC with one TLC system (C + GLC) finds the drugs ranging from 2nd to 7th positions in the list of hits, whereas the inclusion of a second TLC system (AB + GLC) gives a better result with the drugs ranging from 1st to 4th positions. The inclusion of a third TLC system (ABC + GLC) gives a further small improvement. The final search shown in Table 6 shows the combination of a single TLC system (C) with GLC and UVA, where all drugs appear in either 1st or 2nd place in the list of hits. This search would appear to be extremely efficient in terms of the practical work required but is probably slightly biased by the fact that only 24% of the compounds in the computer file have data for system UVA and hence appear in the first block of hits where four systems have been compared. Overall, it is clear that several approaches can be adopted to minimise the amount of analytical work undertaken that can still generate useful information from the search program to assist in the identification of unknowns.

Conclusions

A computerised data base of GLC, TLC and UV spectroscopic information containing over 1600 compounds has been set up. This data file can be interrogated through a dedicated computer program that allows the user to find the best matches for experimental data or to retrieve information for specific compounds or groups of compounds. The system alleviates the many problems associated with the use of the same data in printed tables and will find widespread application for drug screening in forensic toxicology. It is intended that the data base will continue to be modified in the future by the verification of the existing data, by the addition of data to fill existing gaps and by the inclusion of information for new compounds. Further, it is envisaged that data for new types of analytical system (*e.g.*, high-performance liquid chromatographic retention data) will be added in due course.

Readers interested in further details of the computer program described in this paper are invited to contact the authors.

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Statistical Evaluation of Methods Using Headspace Gas Chromatography for the Determination of Ethylene Oxide

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Two methods for the determination of levels of ethylene oxide, both based on headspace gas chromatography but one involving internal and the other external standardisation, have been evaluated by comparing their performances in determinations by three analysts using previously characterised material. Statistical analysis has shown that the methods give results that are generally consistent. There was a significant interaction between one analyst and the external standard method, associated with intermittent high determinations, but there was little evidence of such interactions involving the other analysts. There was also little evidence of any significant difference between the two methods, both of which may be recommended subject to the use of conditions adequate for the complete desorption of ethylene oxide.

Keywords: *Ethylene oxide determination; headspace gas chromatography; statistical analysis*

Several methods have been developed for the determination of levels of ethylene oxide involving colorimetry,[1-3] gravimetry,[4] titrimetry[5] and measurements of the deactivation of enzymes.[6,7] However, methods using gas chromatography (GC) are generally preferred, owing to their potential combination of sensitivity, reliability and rapid performance on a routine basis. Several methods for the GC analysis of solvent extracts have been reported.[8-13] An alternative procedure based on headspace gas chromatography (HGC) has been developed by Romano *et al.*[14] and is widely used. Some modifications to this method have been described, *e.g.*, for calibration using internal standards[15] and for automated multiple headspace extraction.[16]

A fundamental obstacle to validating any method for the determination of residual ethylene oxide is that it is difficult to produce experimental material containing constant levels. These depend on factors including desorption conditions,[17] the nature of the materials[18] and the conditions of storage. In a comparative study of some GC methods and a gravimetric procedure,[19] considerable differences between the results from different laboratories were found, despite the use of similar materials under carefully controlled conditions. The purpose of this work was to evaluate and compare the method of Romano *et al.*,[14] calibrated using an external standard of dilute ethylene oxide in air, with an alternative HGC method[20] calibrated with dilute aqueous solutions and using acetone as an internal standard. These methods have been used to determine residual levels of ethylene oxide in previously characterised material containing 10–120 μg of ethylene oxide per gram of material. To overcome problems associated with the inherent variability of the residual level of ethylene oxide, experimental work was carried out over a short period of time by three analysts working in one laboratory. Sufficient independent determinations were made for meaningful statistical comparisons to be made using the results obtained by all analysts using both methods, thereby testing the mutual and self-consistency of analysts and methods.

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Statistical Design of Experiments

Two samples of material were used, with levels of ethylene oxide of *ca.* 90 μg g^{-1} ("high level") and *ca.* 10 μg g^{-1} ("low level"). Surgical materials were packed in sealed envelopes, each containing ten plastic samples (see below). Prior to the determinations reported below, it was established that, although there was considerable variation in the level of ethylene oxide between envelopes, there was acceptable homogeneity within envelopes.

The experiments were designed to measure effects due to methods, analysts and method - analyst interactions, eliminating the effects of inhomogeneity between envelopes. This was achieved by the matching and random allocation of plastic samples from each envelope between the analysts. However, the design did not eliminate the possible effects of differences between analysts with respect to their familiarity with the two methods.

The experimental design[21] for the "high level" sample (Table 1) required at least 25 envelopes for adequate statistical assessment. Two analysts performed determinations using both methods on the materials in each envelope, using two plastic samples for each determination. In interpreting the results, detailed comparisons were confined to determinations on plastic samples from the same envelope. Combinations of these comparisons for all envelopes determined the average differences between methods for each analyst ($[\bar{x} - \bar{x}_E]_{A \text{ and } B}$), the average differences between analysts for each method ($[\bar{x}_A - \bar{x}_B]_{I \text{ and } E}$) and the interaction effect ($x_A{}^I - x_B{}^I - x_A{}^E + x_B{}^E$), Table 1.

The design adopted for the low level sample involved 12 randomly selected envelopes. Using five plastic samples for each determination, one analysis by each method was performed on the contents of each envelope, and the analysts exchanged methods during the experiment. Again from the differences between determinations for all envelopes, a combined average difference between methods and analysts was obtained. This simplified design, adopted because of constraints on time, did not allow possible significant differences between methods or between analysts to be determined separately, but supported the more extensive experiment on "high level" materials.

Table 1. Experimental design for "high level" material (2 × 2 × 25)

Analyst	Method: Internal standard	Method: External standard	Average result
A	$x^{I}_{1A}, x^{I}_{2A}, \ldots, x^{I}_{25A}$	$x^{E}_{1A}, x^{E}_{2A}, \ldots, x^{E}_{25A}$	$\bar{x}_A$
B	$x^{I}_{1B}, x^{I}_{2B}, \ldots, x^{I}_{25B}$	$x^{E}_{1B}, x^{E}_{2B}, \ldots, x^{E}_{25B}$	$\bar{x}_B$
Average results ..	$\bar{x}_I$	$\bar{x}_E$	

Experimental

Materials

Surgical items of plastic material were sterilised by ethylene oxide and packaged in hermetically sealed aluminium foil envelopes. Each envelope contained ten separate plastic samples, each of *ca.* 0.1 g. Two batches of material were prepared, which, at the time of the experimental work, contained residual levels of ethylene oxide of *ca.* 90 μg g^{-1} (high level) and *ca.* 10 μg g^{-1} (low level). Preliminary tests on individual plastic samples showed that coefficients of variation within single envelopes were generally *ca.* 5% and always less than 10%. It was also established that the optimum conditions for desorbing ethylene oxide from small amounts of this material involved heating at 120 °C for 10 min. Higher temperatures (up to 180 °C) and longer heating times (30 min) had no significant effect on the results.

Ethylene oxide (in 1-ml ampoules) and acetone (analytical-reagent grade) were supplied by Fisons Scientific Equipment. Solutions were prepared using doubly distilled water, pH 5.5–6.0.

Vials (nominal volume 15 ml) were used with Hycar septa or silicone discs and one-piece aluminium seals. The volumes of vials used in the external standard method were calibrated. Internal standard solutions were introduced using 5-μl liquid syringes (SGE 5B), and headspace samples were taken using gas-tight syringes, 1-ml Pressure Lok (internal standard method) or 0.1-ml Hamilton 1710 (external standard method). The heating systems used (120 ± 0.2 °C) were electrically heated aluminium blocks drilled to receive the vials, and air circulating ovens. Gas-chromatographic analyses were performed with Pye 104 and Perkin-Elmer F11 instruments using packed columns: external standard method, Carbowax 20M (10%) on Chromosorb (100–120 mesh), 1.5 m, 120 °C; internal standard method, Chromosorb 101 (80–100 mesh), 1.5 m, 125 °C. All chromatographic peaks were integrated.

Procedure

Internal standard method[20]

Aqueous solutions were prepared for calibration (C) and internal standardisation (S), the former containing ethylene oxide (high level, 3.74 g l^{-1}; low level, 0.374 g l^{-1}) and both containing acetone (high level, 0.30 vol.-%, low level, 0.030 vol.-%). Sealed vials, (a) empty or (b) containing pre-weighed plastic samples, were evacuated, after which 5 μl of (a) solution C or (b) solution S were introduced. Each vial was then placed in a heated block for 10 min, after which, with the vial still in the block, pressure was equalised using a hypodermic needle, which was then removed. A sample of headspace (gas syringe, 1 ml) was taken immediately for analysis, the syringe being allowed to fill, flushed once into the vial and then allowed to refill for 30 s. Between each sampling the gas syringe was dismantled and flushed with nitrogen. Calibration ratios (peak areas of ethylene oxide to acetone) were consistent throughout at both levels and on all chromatographs (ratio = 0.625, coefficient of variation 3.7%). Varying the relative proportions of ethylene oxide and acetone in calibration solutions (C) gave directly proportional changes in the ratio.

Levels of ethylene oxide, x μg g^{-1}, were given by

$$x = \frac{10^6 k}{m}\left(\frac{E}{A}\right)_d\left(\frac{A}{E}\right)_c$$

where E and A are peak areas for ethylene oxide and acetone, respectively, in calibrations (c) and determinations (d), m is the mass of the plastic sample in grams and k is the mass of ethylene oxide introduced in calibrations, *i.e.*, 1.87 × 10^{-5} g (high level) or 1.87 × 10^{-6} g (low level).

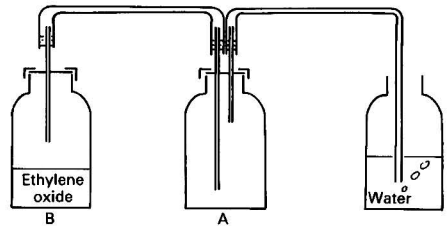

Fig. 1. Preparation of external standards. Air in A is displaced by ethylene oxide vapour from B; the vapour reaches ambient temperature and pressure in *ca.* 20 min

External standard method[14,22]

To prepare the standard, air in a sealed vial (A, Fig. 1) was displaced by ethylene oxide vaporising from liquid (b.p. 11 °C) in a second sealed vial (B). Connections were made via hypodermic needles and PTFE tubing, and the displaced air was led under water. The displacement took 15–20 min, after which the needle admitting ethylene oxide to vial A was removed. The needle from A to exhaust was removed as soon as the pressure had equalised to atmospheric. (Subsequent removal of the needle from B allowed liquid ethylene oxide to be conserved for limited periods.) Finally, a gas syringe was used to transfer 0.1 ml of ethylene oxide vapour from A into a sealed vial, C; the syringe was cleared into C several times. The standard C was allowed to stand for 10 min before use, and was reliable for up to 1 h. Calibrations were carried out before and after each set of determinations, using 0.1-ml samples from C; the syringe was flushed with nitrogen between injections. Calibrations were linear for headspace concentrations resulting from the desorption of 2–30 μg of ethylene oxide. Calibration peak areas were consistent throughout the experiments, with a coefficient of variation of 1.7%.

In determinations, each vial containing weighed plastic samples was placed in an air circulating oven for 15 min. A sample of headspace (0.1 ml) was taken immediately after removing the vial from the oven, to which it was then returned. A second sample was taken as soon as ethylene oxide from the first had been eluted; good agreement was almost always obtained.

Levels of ethylene oxide, x μg g^{-1}, were given by:

$$x = \frac{10^6 M}{m}\cdot\frac{P}{RT}\cdot\frac{vV_d}{V_c}\cdot\frac{E_d}{E_c}$$

where M is the relative molecular mass of ethylene oxide, R is the universal gas constant and T and P are ambient temperature and pressure (all in SI units), v is the volume of ethylene oxide (ml) taken for dilution from vial A and V and E are

Table 2. Determinations of ethylene oxide, "high level"

Envelope No.	Level of ethylene oxide/μg g^{-1} Analyst A IS*	Analyst A ES*	Analyst B IS*	Analyst B ES*	Average	Standard deviation	Coefficient of variation, %
4	98.9	98.6	101.4	101.5	100.1	1.6	1.6
50	60.1	52.4	59.4	61.6	58.4	4.1	7.0
48	85.4	82.4	81.3	85.9	83.8	2.3	2.7
30	79.0	72.9	79.1	85.3	79.1	5.1	6.4
44	24.1	22.3	26.8	29.1	25.6	3.0	11.7
14	91.5	91.4	90.2	99.7	93.2	4.4	4.7
39	68.9	75.8	66.9	72.8	71.1	4.0	5.6
17	86.1	89.9	93.6	88.2	89.5	3.2	3.6
33	75.4	75.5	78.9	78.9	77.2	2.0	2.6
7	87.6	89.5	89.3	93.1	89.9	2.3	2.6
47	69.9	59.5	66.4	61.3	64.3	4.8	7.4
6	103.0	99.4	101.8	101.7	101.5	1.5	1.5
3	109.0	98.0	115.4	109.3	107.9	7.2	6.7
51	89.3	90.7	89.6	93.4	90.8	1.9	2.1
9	86.7	96.6	93.8	101.1	94.6	6.0	6.4
40	73.3	72.6	71.9	76.1	73.5	1.8	2.5
16	80.7	88.0	90.8	96.7	89.1	6.6	7.5
32	72.2	72.3	76.9	83.1	76.1	5.1	6.7
13	66.0	72.3	72.4	67.7	69.6	3.3	4.7
18	71.2	72.8	71.3	77.4	73.2	2.9	4.0
45	75.8	80.7	77.6	88.7	80.7	5.7	7.1
26	63.2	70.0	66.0	77.1	69.1	6.0	8.7
27	72.1	57.5	67.3	71.3	67.1	6.7	10.0
43	51.9	39.8	51.1	48.6	47.9	5.6	11.6
31	79.4	78.5	85.9	83.8	81.9	3.5	4.3
$\bar{x}$	76.8	75.9	78.6	81.3	78.2	4.0	5.6
σ_{n-1}†	17.3	18.8	18.2	18.3	17.8	1.8	2.9

* IS = internal standard method; ES = external standard method.
† σ_{n-1} = standard deviation.

volumes of vials (ml) and peak areas of ethylene oxide, respectively, in calibrations (c) and determinations (d).

Results

Determinations of Ethylene Oxide

In measurements on "high level" material, 25 randomly selected envelopes were used, for each of which two determinations were carried out using each method (Table 2). (Here and in Table 3, results given for the external standard method are the means of duplicate analyses of headspace samples.) Close agreement between the four determinations was obtained for each of 12 envelopes, having coefficients of variation of less than the mean, 5.8%. Taking all results, the means of determinations by the two methods (*e.g.*, for envelope 4, 100.15 μg g^{-1} by the internal standard method and 100.05 μg g^{-1} by the external standard method) did not differ significantly (*t*-test based on 95% confidence, 48 degrees of freedom, $t \geqslant 2.01$ for a significant difference; from the results in Table 2, $t = -0.18$).

In measurements on "low level" material, one determination by each method was carried out on the contents of each of 12 randomly selected envelopes (Table 3). Reasonably close agreement was obtained in all but one pair of determinations, the higher coefficients of variation being due at least in part to the reduced number of determinations per envelope. If the exceptional pair of results is excluded, the mean levels detected by the two methods agree very closely (internal standard method, 11.8 μg g^{-1}; external standard method, 11.7 μg g^{-1}).

Statistical Analysis

As the inherent variations in the level of ethylene oxide, *x*, between plastic samples within single envelopes were expected to be small, the *t*-test was used to examine the paired differences between determinations on materials from the same envelope, thus eliminating the large variation between envelopes.

Table 3. Determinations of ethylene oxide, "low level"

Envelope No.	Level of ethylene oxide/μg g^{-1} IS*	ES*	Average	Standard deviation	Coefficient of variation, %
3	11.0	12.5	11.8	1.1	9.0
4	14.4	15.8	15.1	1.0	6.6
7	10.6	10.8	10.7	0.1	1.3
8	9.5	11.3	10.4	1.3	12.2
12	10.8	9.3	10.1	1.1	10.6
11	10.9	17.3	14.1	4.5	32.1
1	10.1	10.0	10.1	0.1	0.7
2	11.9	10.6	11.3	0.9	8.2
5	12.0	13.3	12.7	0.9	7.3
6	11.7	10.0	10.9	1.2	11.1
9	16.4	15.9	16.2	0.4	2.2
10	11.3	9.7	10.5	1.1	10.8
$\bar{x}$	11.7	12.2	12.0	1.1	9.3
σ_{n-1}†	1.9	2.8	2.1	1.1	8.2

* IS = internal standard method; ES = external standard method.
† σ_{n-1} = standard deviation.

"High level" determinations

Six sets of paired differences were calculated from the four sets of determinations, C_1 to C_6 in Table 4, giving various over-all differences between analysts and/or methods. The

differences were taken as positive if determinations by the internal standard method or by analyst A were higher than those by the external standard method or analyst B. From the means of these differences for individual envelopes [Fig. 2(*a*)], the over-all average of $-2.7\ \mu g\ g^{-1}$ shows that determinations by analyst B and/or using the external standard method tended to be higher. The mean range of differences, $13.2\ \mu g\ g^{-1}$ [Fig. 2(*b*)], was *ca.* 16% of the average level of ethylene oxide over all determinations. Application of 95% confidence limits to the results in Fig. 2 shows that the results from envelopes 47,

Table 4. Comparison of pairs of differences in determinations from 25 individual envelopes, "high level" material. Analysts are identified as A and B and methods as ES (external standard), IS (internal standard). Comparisons refer to differences between determinations on materials from single envelopes: IS − ES and A − B

Comparison No.	Analyst A IS	Analyst A ES	Analyst B IS	Analyst B ES	Net difference measured	*t**	Significant (S) or non-significant (NS)† 5% level	1% level	0.1% level
C_1	x		x		Analyst	−2.24	S	NS	NS
C_2	x			x	Analyst - method	−3.88	S	S	S
C_3		x	x		Analyst - method	−2.35	S	NS	NS
C_4		x		x	Analyst	−5.79	S	S	S
C_5	x	x			Method	0.65	NS	NS	NS
C_6			x	x	Method	−2.72	S	NS	NS

* Student's *t* statistic.[23,24]

† The hypothesis tested is that differences C_1, etc., do not differ significantly from zero. S at the 5% level is suggestive, at the 1% level significant and at the 0.1% level highly significant.

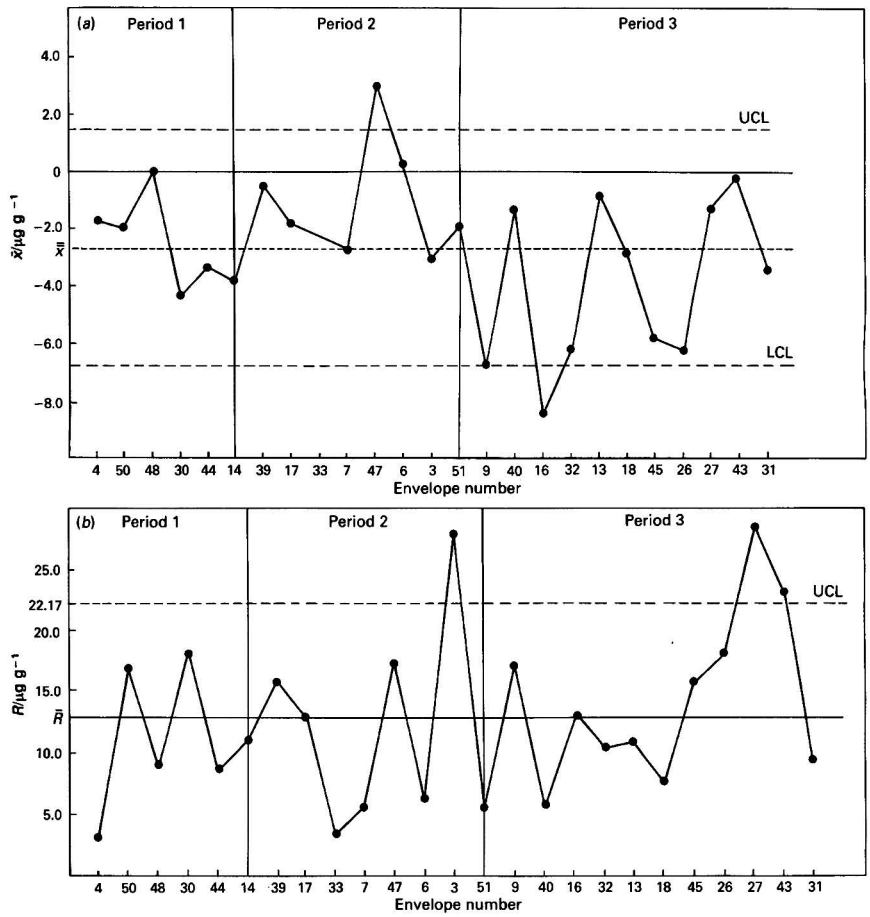

Fig. 2. Statistical analyses for "high level" samples. (*a*) Variation in the means of determination differences ($\bar{x}$); and mean of all the determination differences ($\bar{\bar{x}}$); and at 95% confidence limits (UCL, LCL). (*b*) Variations in the ranges of determination differences (R); mean of all the determination differences ranges ($\bar{R}$); 95% confidence limit (UCL)

16, 3, 27 and 43 fall outside the confidence limits, indicating, *e.g.*, some operational malfunction or exceptional variability of the material.

Individual comparisons of the results of analyst A using the two methods (C_5, Table 4) showed consistency (difference not significant) at all levels of confidence. Comparisons of the results of analyst B using the internal standard method with those of analyst A using both methods (C_1 and C_3, respectively) were suggestive of significant differences (significant at the 5% level), but this was not confirmed at higher levels. However, separate comparisons of both sets of results of analyst A with the results of analyst B using the external standard method (C_2 and C_4, respectively) showed differences that were significant even at the 0.1% level. Hence there appears to have been some significant interaction between analyst B and the external standard method, which is also likely to have contributed to the suggestive result of comparison C_6. From the mean differences [Fig. 2(*a*)], the major contributions to the significance of comparisons C_2 and C_4 were from envelopes 9, 16, 32, 45 and 26 in which the results of analyst B using the external standard method were 10–20% higher than the means. It is notable that most of these determinations were carried out during the third experimental period during which the largest number of analyses was performed. Taking the results as a whole, and excepting the intermittent interaction between analyst B and the external standard method, there is little evidence of a significant difference between the two methods.

"Low level" determinations

A single set of paired differences reflected the combination effects of differences between methods and interaction between analysts and methods. Only one difference, envelope 11 lay outside the 95% confidence limits (−3.26 to +5.54 μg g^{-1}) but has not been excluded from further statistical analysis. The value of t based on 11 degrees of freedom, −1.97, showed that there was no significant difference between the results obtained by the two methods. Although the sample size was small, this result provides useful support for the deductions made from determinations on "high level" material.

Discussion

The aims of this study were to establish the mutual and self-consistency of the two headspace GC methods for determining residual levels of ethylene oxide in plastic samples. Because of the nature of the analytical problems, experimental work was performed over a short period of time under intensive conditions. The analysts had previous experience of the methods before the experimental work was carried out, but were not equally familiar with both. These practical limitations effectively strengthen the positive conclusions reached. The very close agreement between the over-all mean levels, for both "high level" and "low level" samples, obtained by the two methods strongly supports their reliability in routine use.

However, although most independent determinations corresponded closely within single envelopes, one was out of line in 16% of cases and in a further 17% a broad spread of determinations was obtained. The statistical analysis has shown that most of these discrepancies were associated with high results being obtained intermittently by one analyst using the external standard method. For both methods, calibration factors were consistent throughout, so that instrumental factors are unlikely to have caused the above effects. There were no discernable variations in operating procedures during the different periods or between analysts.

In comparing the external standard and internal standard methods, it is noted that the pre-determined conditions for desorbing ethylene oxide from the plastic samples were very similar in both methods. The results show firstly that the two calibration procedures are mutually consistent and secondly that, whether desorption takes place under water vapour (internal standard method) or air (external standard method), the only significant parameters are temperature, time and the nature of the material. A possible uncertainty associated with the sampling of headspace vapours by the external standard method lies in assuming that the vapour does not cool appreciably while being drawn into the syringe. The present results generally justify this assumption, but partial cooling of headspace vapours in the gas syringe could have been responsible for the "high" results obtained by one analyst. The strategy of allowing vials to cool before sampling the headspace is not recommended.

In comparing the two methods for regular use, it is noted that the external standard method involves fewer manipulations with syringes, but may be subject to intermittent operator - method interactions. However, the effect of these interactions is relatively small (<20%) and is on the side of safety, *i.e.*, high results are obtained. No such interactions were found with the internal standard method, but the procedure for this method is more complex. In general, both methods reliably determine concentrations of ethylene oxide in headspace vapours above small amounts of absorbent material (<0.5 g). Provided that optimum conditions for the desorption of ethylene oxide are used, determinations using either method are reliable to within *ca.* 3% for residual levels of 90 μg g^{-1} and 7% for residual levels of 10 μg g^{-1}.

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Determination of Ethylene Glycol Using Periodate Oxidation and Liquid Chromatography

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The coupling of a simple pre-derivatisation reaction step with high-performance liquid chromatography permitted the determination of ethylene glycol at levels from 12 to 100 p.p.m. Ethylene glycol was oxidised by periodate (IO_4^-) to form formaldehyde and iodate (IO_3^-). The iodate was retained on a polymeric anion-exchange column for about 8 min and the resulting peak was used to quantitate the initial ethylene glycol in the sample. Using UV absorbance at 254 nm, the detection limit for iodate corresponded to about 12 p.p.m. of ethylene glycol. A simple but effective extraction scheme was developed to permit the determination of ethylene glycol in either new or used oil samples.

Keywords: *Ethylene glycol determination; periodate oxidation; high-performance liquid chromatography*

The oxidation of ethylene glycol and other polyhydric alcohols containing adjacent hydroxy groups, such as sugars, by periodate is a well established qualitative and quantitative method.[1]

$$HOCH_2(CHOH)_nCH_2OH + (n + 1)HIO_4 \rightarrow (n + 1)HIO_3 + 2HCHO + nHCOOH + H_2O$$

Classically, the remaining unreacted periodate is determined iodimetrically or using arsenite and related to the initial amount of diol in the sample. These titrimetric procedures are suitable for millimolar amounts of polyhydric alcohols but not trace levels.

A number of instrumental detection methods in conjunction with the periodate oxidation step have been proposed in an effort to speed the analysis or lower the detection limits for the determination of ethylene glycol. The perchlorate ion-selective electrode has been used to monitor the decrease in periodate.[2,3] In addition to being an indirect method, it was not particularly sensitive, being applied only to ethylene glycol in antifreeze solutions. Oles and Siggia[4] have precipitated the iodate formed with Ag^+ and determined the metal by atomic-absorption spectrometry. Although the detection limit was about 12 p.p.m., the time for precipitation at low concentrations was long and washing of the precipitate was required at a temperature below 0 °C. Probably the most common methods are spectrophotometric. The formaldehyde formed from the IO_4^- oxidation has been reacted with either *p*-fuchsin,[5] chromatropic acid[6] or methyl-2-benzothiazolinone hydrazone[7] to form coloured dyes. Although the detection limits are in the parts per million range, decolorisation of the dye reagent, the use of strong solvents such as sulphuric acid or lengthy reaction times can limit the usefulness of these methods.

Surprisingly, even with the advent of high-performance liquid chromatography (HPLC), chromatography in conjunction with the periodate oxidation step has generally been ignored. Classical low-pressure anion-exchange columns have been used for the separation of millimolar levels of IO_3^- from IO_4^-.[8,9] However, periodate oxidation and subsequent separation of iodate by anion-exchange HPLC offers the possibility of the rapid analysis of certain diol samples using simple reagents and instrumentation. We report such a method here for the determination of ethylene glycol in oil samples. This analysis is important for the detection of coolant leaks into the engine that could markedly affect engine lubrication and performance. In addition, a non-tedious scheme for the extraction of ethylene glycol from oil was developed and found to be effective when used with the periodate HPLC method.

Experimental

Apparatus

The HPLC system was assembled from various components as follows. The high-pressure Milton-Roy Model 396 pump (Laboratory Data Control, Riveria Beach, FL, USA) was modified with a tee-configuration pulse damper consisting of a 52 cm × 4.6 mm i.d. stainless-steel tube. The injector, equipped with a 50- or 100-µl sample loop, was a Rheodyne Model 7010 with a Model 7011 loop filler port (Rheodyne, Berkeley, CA, USA). The separations were effected with a 2 cm × 2 mm i.d. Ionguard G-100 pre-column and a 5 cm × 3.2 mm i.d. ION-100 analytical column (Interaction Chemicals, Mt. View, CA, USA). Both columns were packed with 10-µm quaternary ammonium derivatised polystyrene - divinylbenzene resin particles. The capacity of this strong anion-exchange resin was about 0.1 mequiv. g^{-1}. The columns were water-jacketed and maintained at 38 °C. An Altex Model 153 UV detector (Altex, Berkeley, CA, USA) was used to monitor the column effluent at 254 nm. Peaks were recorded with a Fisher Recordal Model 5000 (Houston Instruments, Austin, TX, USA). In addition, the chromatograms were digitised by an Apple 2+ minicomputer (Apple, Cupertino, CA, USA) equipped with a 12-bit analogue-to-digital converter (Interactive Microware, State College, PA, USA).

Chemicals

All solutions were prepared in doubly distilled, de-ionised water by dissolution of analytical-reagent grade chemicals and were kept in Pyrex glassware. Stock solutions of sodium metaperiodate were stored in amber-glass bottles in the dark and were stable for at least 1 month if protected from light and organic material.[10] For the periodate column packing reactions, 10 µm RSiL anion-exchange silica (Alltech Associates, Deerfield, IL, USA) and 50–100-mesh Dowex 2-X8 anion-exchange polymeric resin (J. T. Baker, Philipsburg, NJ, USA) were used.

Procedure

All periodate - ethylene glycol reactions were carried out in 50-ml amber-glass bottles at room temperature and at pH 4 as recommended previously.[11]

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Optimisation of sodium metaperiodate concentration

A sample was prepared containing 35.8 mM of ethylene glycol and 1 ml of this sample solution was reacted with 9 ml of a 0.5 M acetate buffer (pH 4.0), which varied in sodium metaperiodate concentration. After the solutions had been allowed to react for 30 min, the samples were injected into the HPLC system. Peak-area determinations were made using a Micromeritics Model 740 Control Module (Micromeritics Instrument Co., Norcross, GA, USA).

Kinetic study

A sample was prepared containing 12.9 mM of ethylene glycol and 1 ml of this sample solution was reacted with 9 ml of a 0.5 M acetate buffer (pH 4.0), in which the sodium metaperiodate concentration was 5.74 mM. The solution was allowed to react for periods of 5, 10, 20, 30 and 330 min. The samples were then injected into the HPLC system and analysed as described for the previous study.

Calibration graph

A 2-ml aliquot, of various concentrations, was placed in a 10-ml calibrated flask. A 1-ml aliquot of a 55.1 mM solution of sodium metaperiodate dissolved in 0.5 M acetate buffer (pH 4) was then added to each flask and the flasks were diluted to the mark with water. After the reaction had proceeded for 15 min, the samples were analysed with the HPLC system. Peak areas were determined using Autovideogration software (Heyden and Sons, Philadephia, PA, USA).

Recommended procedure for oil samples

A 4.0-ml of oil, spiked with the desired amount of ethylene glycol, was diluted with 10–20 ml of hexane. The extraction was carried out once using 4 ml of water. After separation, the aqueous phase was extracted with three 10-ml portions of chloroform. After decanting the organic phase, the aqueous phase was allowed to react for 15 min with a 2-ml aliquot of a 34.2 mM solution of sodium metaperiodate in 0.5 M acetate buffer at a pH of 4.0. The samples were then analysed as described for the calibration graph.

Results and Discussion

Chromatographic Separation of IO_3^- and IO_4^-

Initially, the study and optimisation of the separation of periodate and iodate by anion-exchange HPLC was of concern. Our first attempt was to use a 15 cm × 4.1 mm i.d. column packed with the anion-exchange silica. An early chromatogram of an iodate and periodate mixture using this column is shown in Fig. 1(*a*). The first peak at approximately 7.5 min was due to the iodate and the broad peak at approximately 50 min was due to the periodate. As the periodate was strongly retained, we thought we could make up to four injections before the first periodate peak would be eluted. After the fourth injection, the mobile phase would be switched to 0.1 M acetate (pH 6.5), so that the periodate could be eluted quickly in about 13 min. However, after 30 injections using this procedure, a dramatic change in retention time occurred, as shown in Fig. 1(*b*): now the periodate had a retention time of only 24 min and it continued to decrease with further injections.

To determine whether the periodate was degrading the silica or the quaternary ammonium functional group, the following experiments were run. A 0.28-g sample of the silica anion exchanger was allowed to react for 24 h with 25 ml of a 400 p.p.m. IO_4^- solution prepared using the pH 4 acetate buffer. On titration of this sample in the hydroxide form with HCl, no end-point could be observed. The unreacted packing could be titrated in a similar fashion, indicating a capacity of 0.2 mequiv. g^{-1}. The same experiment was carried out using a

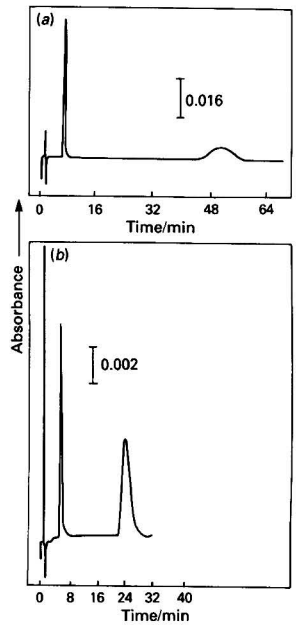

Fig. 1. Separation of IO_3^- and IO_4^- on the RSil silica anion-exchange column. (*a*) Injection of a 350 p.p.m. IO_3^- and a 382 p.p.m. IO_4^- mixture. (*b*) After 30 injections of a 69 p.p.m. IO_3^- and 382 p.p.m. IO_4^- mixture. Mobile phase: 25% methanol - 75% 0.01 M acetate, pH 5.7; flow-rate, 1.5 ml min^{-1}; injection volume, 100 µl; temperature, ambient

Dowex polymeric anion-exchange resin. Titration of the OH^- form of either the IO_4^- reacted or the unreacted resin yielded the same end-point and a capacity of about a mequiv. g^{-1} could be calculated. These data and the chromatographic results led us to believe that periodate was probably degrading at least the silica supporting accessible ion-exchange groups.

Therefore, all subsequent chromatography of IO_3^- and IO_4^- was investigated with the Interaction polymeric anion-exchange columns using a 0.0025 M sodium acetate (pH 5.4) mobile phase at a flow-rate of 0.5 ml min^{-1}. On injection of a 200 p.p.m. IO_3^- and a 382 p.p.m. IO_4^- standard solution, the only peak visible at approximately 7.5 min was due to the iodate, even after waiting 5 h. The periodate ion was considered to be irreversibly retained under these mobile phase conditions. A similar result has been observed previously using low-pressure, high-capacity anion-exchange clean-up columns.[9] Further injections of the iodate and periodate standards indicated no change in the HPLC column performance with time. However, an increase in ionic strength of the mobile phase to 0.2 M citrate could elute the IO_4^- if desired.

Reaction Optimisation and Calibration Graph

Experiments were carried out to ascertain the effects of concentration of IO_4^- and reaction time for the periodate oxidation of ethylene glycol. The results shown in Fig. 2 indicate that the maximum sensitivity, for a reaction time of 30 min, was obtained when the molar ratio of sodium periodate to ethylene glycol was approximately 4. Although over-oxidation is not a problem for simple organic compounds such as ethylene glycol, a modest stoicheiometric excess of periodate is generally recommended.[11] The results shown in Fig. 3 indicate that the oxidation of ethylene glycol by sodium periodate under these conditions is essentially complete after

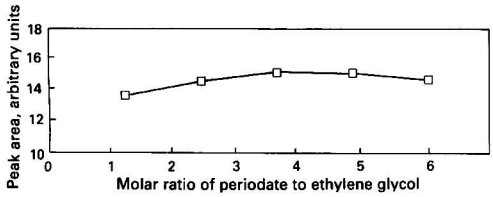

Fig. 2. Formation of IO_3^- determined by HPLC as a function of the reactant IO_4^- to ethylene glycol ratio

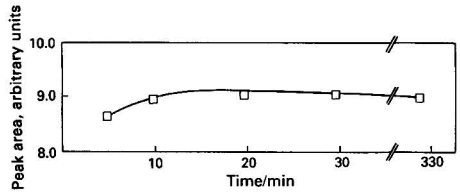

Fig. 3. Formation of IO_3^- determined by HPLC as a function of reaction time

Table 1. Peak-area measurements for iodate formed as a function of ethylene glycol in the original sample

Ethylene glycol, p.p.m.	Mean peak area, arbitrary units	Standard deviation ($n = 3$)
0.0	33.5	1.9
12.4	48.5	6.4
16.6	78.4	8.5
33.2	140.0	3.9
49.7	184.3	5.7
66.3	258.3	18.2
82.9	311.3	21.1
99.5	374.3	18.7
124.4	428.1	—

10 min. A reaction time of 15 min was used in all subsequent work. However, the formation of iodate was independent of longer reaction times, permitting a fairly large batch of samples to be oxidised simultaneously for subsequent HPLC analysis.

Using these optimum conditions, representative IO_3^- peak areas for a range of ethylene glycol samples were generated, as shown in Table 1. A calibration graph was obtained by plotting the area of the iodate peak (arbitrary units) against the concentration of ethylene glycol in parts per million. A relative standard deviation (RSD) for the slope of 2.4% could be calculated for points between 12 and 100 p.p.m. Reasonable linearity was indicated by a correlation coefficient of 0.9987. However, deviation of the straight line was observed at 125 p.p.m., as shown in Table 1. Essentially the same linear range using a spectrophotometric method has been reported previously.[7] The RSD for the eight sets of points (triplicate measurements) ranged from 2 to 13% and the average value was 6.8%. This reproducibility was about twice that of the previously reported atomic-absorption spectrometric method,[4] but was certainly not unreasonable for a chromatographic method.

The detection limit was considered to be about 12.4 p.p.m. based on the precision of this average peak area using the *t*-test at the 90% confidence level when compared with the blank. This detection limit was considered to be good as the molar absorptivity of iodate was found to be only 187 l mol^{-1} cm^{-1} at 254 nm. Use of a variable-wavelength detector set at 220 nm could conceivably lower the detection limit based on the increase in molar absorptivity by a factor of 10. However, the acetate mobile phase would also absorb at this wavelength.

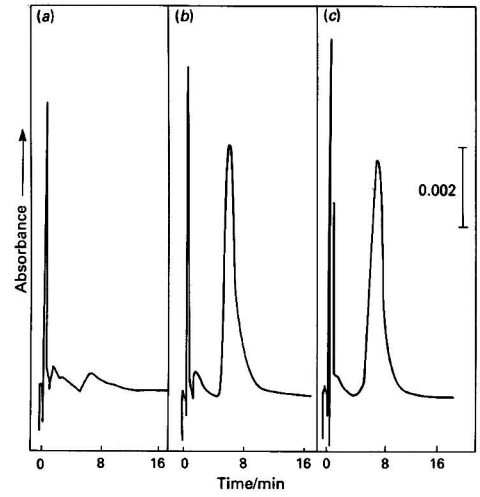

Fig. 4. Chromatograms representing (*a*) used motor oil after extraction, (*b*) 137.7 p.p.m. of ethylene glycol standard and (*c*) used motor oil spiked with 137.7 p.p.m. of ethylene glycol after extraction. Mobile phase, 0.0025 M acetate, pH 5.4; flow-rate, 0.5 ml min^{-1}; injection volume, 50 µl; temperature, 38 °C

Analysis of Automotive Samples

The method was applied to the determination of ethylene glycol in various automotive samples. On simple dilution of antifreeze solutions, the ethylene glycol was easily assayed and found to range from 50 to 80% in samples taken from a variety of motor vehicles. However, it was the determination of trace levels of ethylene glycol in a complicated matrix such as motor oil that was of special interest. To carry out these analyses, extraction of the ethylene glycol from the oil was necessary to prevent fouling of the column. Previous methods of extraction were tedious, involving the slow rotation of the oil sample in a vial with water for at least 1 h. We found a dual organic solvent extraction system was rapid and simple, but still efficient. The oil sample was diluted with hexane to lighten the colour of the oil and permit the detection of the water - oil interface. In addition, hexane was found to minimise emulsion formation during the extraction process. After the ethylene glycol had been extracted into the aqueous phase, this layer was extracted with chloroform to remove any possible surfactants initially present in the oil sample. The total time for this extraction process was no longer than 15 min.

This clean-up procedure was efficient for real oil samples that had been spiked with ethylene glycol. In Fig. 4, the resultant chromatograms for (*a*) an extracted unspiked used oil sample, (*b*) an extracted 137.7 p.p.m. standard of ethylene glycol and (*c*) an extracted used motor oil sample previously spiked to 137.7 p.p.m. with ethylene glycol are presented. A recovery of 98.8% was realised in this instance. Comparison of an extracted ethylene glycol spiked oil sample with a non-extracted ethylene glycol standard gave a recovery of 104%. The probable reason for the recovery being higher instead of lower was the pre-concentration caused by the extraction procedure with a slight volume change. The extraction procedure was used on subsequent samples and standards to ensure the best accuracy.

The results shown in Table 2 indicate the accuracy and precision of the method for a variety of new and used oil

Table 2. Recovery of ethylene glycol from oil

Sample	Amount added, p.p.m.	Amount found ± standard deviation, p.p.m.
New oil A:		
1	137.7	138.8 ± 1.3 (n = 3)
2	68.8	66.9
3	34.4	35.2
4	20.7	24.9
New oil B:		
1	89.5	84.8
2	55.1	53.9
Used oil from gasoline engine:		
1	137.7	136.1
2	92.3	93.9 ± 1.9 (n = 5)
3	42.6	42.3 ± 0.8 (n = 5)
Used oil from diesel engine:		
1	68.9	67.8
2	34.4	34.2
3	0.0	23.6 ± 0.9 (n = 5)
4	42.6	41.8 ± 1.4 (n = 5)

samples at different concentrations. Most recoveries of ethylene glycol from oil [(p.p.m. found/p.p.m. added) × 100] ranged between 95 and 99% with an over-all average of 101%. The RSD values for the multiple-assayed samples ranged from 1 to 4%. Unexpectedly, used motor oil was found to be easier to analyse owing to the smaller blank. The blank peak area for extracted unspiked used oil samples was generally the same as a reaction standard blank. One expected exception, as shown in Table 2, was diesel oil sample 3, which came from an engine with over 100000 miles of use. However, the blank peak area for new oil samples was about a factor of 2 larger. These results could be explained by likely engine degradation of oil additives that may also react with periodate. The used diesel oil samples, although darker in colour and more viscous, presented no special problems.

This extraction system and HPLC method for ethylene glycol in oil took over-all about 25 min per sample, as opposed to almost 2 h per sample for a previously reported dye method.[7] In addition, the recovery and reproducibility are essentially the same as those in the spectrophotometric method.[7] Finally, even after about 100 injections, no change in peak retention or shape had been observed using the combined guard and analytical polymeric anion-exchange columns. It is likely that the HPLC conditions could be easily adapted to the determination of a variety of organic compounds such as amino alcohols and polyhydric alcohols in certain matrices.

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Concentration of Aqueous Solutions of Salts Using Silica Gel Treated with a Mixture of Liquid Anion Exchanger and Eriochrome Black T Prior to Analysis for Trace Amounts of Bivalent and Trivalent Metals*

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Silica gel and silanised silica gel retain very strongly ion pairs formed with Aliquat 336 and Eriochrome Black T; the dyestuff is not eluted from the sorbent even with 5–6 M hydrochloric acid or 0.6–1 M perchloric acid. The strong retention of many bivalent and trivalent metals on sorbents prepared in this way was utilised for the pre-concentration of trace amounts of copper, lead, iron(III) and alkaline earth metals from water and from aqueous solutions of analytical-reagent grade sodium, potassium and ammonium salts. The retained metals were eluted from the column with dilute perchloric acid and determined by atomic-absorption spectrometry. It was found that at least a 250-fold concentration of trace amounts of the retained metals can be achieved. The sorbent was also applied to the purification of sodium, potassium and ammonium salts; it was found on the basis of anodic-stripping voltammetry that the concentrations of lead, cadmium and zinc in the eluates were lower than 10^{-9} M.

Keywords: *Eriochrome Black T; extraction chromatography: trace metal pre-concentration; sodium, potassium and ammonium salt purification*

Eriochrome Black T is commonly used as an indicator in complexometric titrations[1] and as a chromogenic reagent for the spectrophotometric determination of magnesium.[2] The extraction of anionic complexes of magnesium with Eriochrome Black T with solutions of trioctylamine or quaternary alkylammonium salts in chloroform has been utilised for the extraction - spectrophotometric determination of this metal.[3,4] It was found earlier that silica treated with a mixture of an alkylammonium salt and some sulphonated chelating agents for metals can be applied to the concentration of metals from very dilute aqueous solutions[5,6] and for the separation of some metal ion mixtures.[6,7] Eriochrome Black T is quantitatively extracted from acidic and alkaline aqueous solutions with Aliquat 336 in chloroform and the organic solution of this dyestuff is stable,[8] in contrast to the aqueous solution of Eriochrome Black T. On the other hand, the very strong retention of Eriochrome Black T on cellulose treated with Aliquat 336 or trioctylammonium chloride[9] suggested the strong sorption of an ion pair formed by this dyestuff and alkylammonium cations also on silica gel and the possibility of utilising the sorbent prepared in this way for the concentration of bivalent and trivalent metals from aqueous solutions and the separation of these metals from alkali metal and ammonium ions, which cannot form complexes with Eriochrome Black T.

Experimental

Materials

Eriochrome Black T (POCh, Gliwice, Poland) was used without further purification. Aliquat 336 (Fluka, Buchs, Switzerland) containing 93.3% *m/m* of quaternary alkylammonium chloride was purified as described previously.[6] Solutions of Suprapur perchloric acid (Merck, Darmstadt, FRG) were used for elution. Silica gel (0.063–0.2 mm) and silanised silica gel (Merck) were used as supports for the stationary phase.

Aqueous metal ion solutions were prepared by dilution of Titrisol standard metal salt solutions (Merck). All other reagents were of analytical-reagent grade. Doubly distilled water was usually used, except for the concentration of trace amounts of magnesium and calcium, the determination of trace amounts of metals in sodium carbonate and the concentration of metals in water samples, when doubly distilled water from quartz apparatus was used (containing less than 10^{-8}% of metal ions).

Apparatus

A single-beam Pye Unicam SP 192 atomic-absorption spectrometer was used for the determination of the majority of the investigated metals by atomic-absorption spectrometry (AAS); only barium was determined by flame emission spectrometry. Aluminium and chromium were determined spectrophotometrically with a Specord M 40 spectrophotometer (Zeiss, Jena, GDR). In a few experiments lead, cadmium and zinc were also determined in the eluate by anodic-stripping voltammetry (ASV) with a voltammetric analyser (produced in the Department of Analytical Chemistry, Maria Curie-Skłodowska University, Lublin). A graphite electrode, impregnated with epoxy resin and coated with a mercury film *in situ* [the concentration of mercury(II) ions in the sample solution was 2.5×10^{-5} M] was used as a working electrode with a working area of 12.5 mm^2. The details of the preparation of this electrode were described by Sykut *et al.*[10] pH measurements were made with Mera-Elwro N 517 direct-reading pH meter with a glass - calomel electrode assembly.

Procedure

The impregnating solution was prepared by shaking a 0.1 or 0.08 M solution of Aliquat 336 in chloroform with an equal volume of a freshly prepared 0.02 M aqueous solution of Eriochrome Black T. After separating the phases, the organic phase was filtered through a cellulose filter.

Silica gel or silanised silica gel was impregnated with the chloroform solution of Aliquat 336 + Eriochrome Black T as described previously.[6]

Small glass columns (110 × 15 mm) dry-packed with 1 g of the sorbent (containing 23 or 46 μmol of Eriochrome Black T and an appropriate excess of alkylammonium chloride) were

* Based on a paper presented at Euroanalysis V, Krakow, August 26–31, 1984.

used for the determination of the capacities of the sorbent towards metal ions, for the concentration of impurities in sodium carbonate and for the concentration of trace amounts of metals in water samples. Identical columns packed with 0.3 g of the sorbent were used for the concentration of bivalent metals and iron(III) from analytical-reagent grade sodium nitrate, potassium chloride, potassium nitrate, ammonium nitrate and ammonium chloride. Glass columns (290 × 8 mm) dry-packed with 5 g of the sorbent were used for the determination of elution curves for magnesium, calcium, strontium and barium.

After the column had been washed with 5 or 10 ml of doubly distilled water or with an appropriate buffer solution, an appropriate volume of alkali metal salt or ammonium salt solution or a water sample was passed through the column. The column was subsequently washed with 5 or 10 ml of doubly distilled water and the retained metals were eluted with dilute solutions of perchloric acid, 5-ml fractions usually being collected. Elution under hydrostatic pressure (head pressure 380 mmH_2O) was used. The mean flow-rate was about 1 ml min^{-1}.

Results and Discussion

The blue HL^{2-} anion from Eriochrome Black T forms coloured complexes with many metal ions in neutral or alkaline aqueous solutions. As an anionic complex of magnesium with this dyestuff is strongly extracted from alkaline solution with salts of high relative molecular mass amines in chloroform, it was expected that metals that form more stable complexes with Eriochrome Black T (for instance, copper, zinc and lead[11,12]) would also be retained on silica treated with a mixture of Aliquat 336 and Eriochrome Black T from weakly acidic solutions. Therefore, the sorption of some metal ions from acidic and alkaline aqueous solutions was investigated.

Retention of Eriochrome Black T

Preliminary experiments indicated weak adsorption of Eriochrome Black T on silica gel or silanised silica gel in the absence of alkylammonium salts. On the other hand, although solvent extraction data indicated the quantitative extraction of Eriochrome Black T with a solution of the same molarity of Aliquat 336 in chloroform, the dyestuff was partially eluted with water from silica gel treated with an organic solution of Eriochrome Black T. It was found that the molar ratio of Aliquat 336 to Eriochrome Black T must be at least equal to 4:1 in order to prevent elution of the complexing reagent from the sorbent with water. Some sorbents prepared by impregnation of silica gel with mixtures of Aliquat 336 and Eriochrome Black T in chloroform are listed in Table 1.

Although Eriochrome Black T is more strongly retained on silanised silica gel (the dyestuff was not eluted from the sorbent even with 1 M perchloric acid) than on silica gel 100, in further experiments both supports were used: sorbent A for the pre-concentration of bivalent and trivalent metals before their determination and sorbent D for the purification of sodium, potassium and ammonium salts.

The strong retention of Eriochrome Black T is important for the elution of metals from the column and for regeneration of the sorbent with dilute solutions of mineral acids.

Capacity of the Sorbent

It was found that alkali metal and ammonium ions are not retained on silica coated with a mixture of Aliquat 336 and Eriochrome Black T, whereas magnesium, calcium, strontium, barium, aluminium, lead, silver, copper, mercury(I), mercury(II), zinc, cadmium, chromium(III), manganese, iron(III), cobalt and nickel are strongly retained. Although the phenolic group of the dyestuff cannot be dissociated in very acidic solutions (pH 1–3), the experimental results reported in Table 2 indicate that all the investigated metals were retained by the sorbent from aqueous solutions of pH 3 and iron(III), cobalt, nickel and copper even at pH 1.

The capacity of the sorbent towards all the metal ions investigated increases with increasing pH of the aqueous solution, and also depends on the amount of chelating reagent in the liquid stationary phase (compare the results obtained on

Table 1. Retention of Eriochrome Black T on various samples of the sorbent

Sample	Amount of Aliquat 336/ μmol g^{-1}	Amount of Eriochrome Black T/ μmol g^{-1}	Concentration of acid eluting Eriochrome Black T/M	
			HCl	$HClO_4$
A*	115	23	>1	—
B	115	23	>5	>0.5
C	230	46	>6	>0.6
D	184	46	>0.7	>0.02
E	92	23	>0.5	>0.01

* Silanised silica gel.

Table 2. Capacities of sorbent D and sorbent A (values in parentheses)

Metal	Capacity/μmol g^{-1}				
	pH 1.0	pH 2.0	pH 3.0	pH 6.0	pH 9.0
Mg	0	(2.5)	18 (13)	30 (22)	40 (36)
Ca	0	(3.1)	10 (6.5)	16 (12)	25 (20)
Ba	0	3.2	9.8	18	26
Al	0	—	0.2	34	37
Pb	0	(0.2)	4.1 (2.1)	4.4 (3.8)	— (4.5)
Cu	3.9 (1.9)	(5.9)	8.7 (7.8)	14 (8.5)	16 (10)
Ag	0	4.4	4.5	4.6	4.7
Zn	0	—	2.9	10	15
Cd	0	—	3.9	7.4	8.9
Hg(I)	0	0.1	0.4	4.2	10.5
Hg(II)	0	0.1	0.2	3.6	8.4
Cr(III)	0	0.05	0.3	18	—*
Cr(VI)	0	0	0.3	6.3	12
Mn(II)	0	—	7.3	10	18
Fe(III)	14 (8.3)	(11)	—*	—*	—*
Co	5.0	—	11	15	17
Ni	3.4	—	8.3	12	17

* Metal hydroxide precipitate.

sorbents A and D). It is difficult to relate the retention of metals to the stability constants of metal complexes with Eriochrome Black T; although calcium forms a weak complex, the capacity of the sorbent for calcium at pH 6 and 9 was higher than that for copper and lead, which form more stable complexes with Eriochrome Black T. On the other hand, the retention of all the metals from acidic solutions seems to be interesting from the practical viewpoint.

Elution of Retained Metals

It was found that all the investigated metals retained by the sorbent can be eluted with dilute perchloric acid. As the stability constants for complexes of alkaline earth metals with Eriochrome Black T decrease with increasing radius of the cation, we tried to separate these metals retained in slightly alkaline aqueous solution (pH = 8) by stepwise elution with perchloric acid. Preliminary experiments performed on the column packed with 5 g of sorbent A after passing a Ba - Sr - Ca - Mg mixture containing 0.1 mg of each metal ion indicated that all the metals were quantitatively eluted with 0.01 M perchloric acid without separation, whereas even 50 ml of 0.005 M perchloric acid was not sufficient for elution. In further experiments performed with the same column it appeared that all alkaline earth elements were quantitatively eluted with 50 ml of 0.0075 M perchloric acid.

Although magnesium is more strongly retained than other metals (Fig. 1), the differences in retention for alkaline earths are too small for the separation of their mixture. Perchloric acid (0.5 M) was usually used for elution in further experiments in order to secure complete elution of the metals retained from distinctly acidic solutions (iron and copper) and to reduce the volume of the eluate.

Some experiments were performed on trace concentrations of calcium and magnesium. Volumes of 200 ml of a mixture of calcium and magnesium chlorides (the concentration of each metal ion was from 1 to 100 p.p.b., pH 6) were passed through the column, which was subsequently washed with 5 ml of doubly distilled water and then the retained metals were eluted with 2 ml of 0.01 M perchloric acid and determined by AAS. Linear relationships between the concentration of calcium and magnesium and the absorbance were found for concentrations of both metals between 5 and 100 p.p.b. (see Fig. 2).

In other similar experiments a volume of 500 ml of a solution of calcium and magnesium chlorides each at a concentration of 1 p.p.b. was passed through the column. It was found that at least a 250-fold concentration of trace amounts of both metals is possible.

Small columns packed with 0.3 g of the sorbent were used for the concentration of iron, copper, lead, calcium and magnesium present as impurities in some analytical-reagent grade reagents. Volumes of 50 ml of 1% *m/V* solutions of metal chlorides or nitrates (the pH of solutions was between 6.7 and 6.8 for sodium or potassium salts or 5.5 for ammonium salts) were passed through the column and the retained metals were eluted with 5 ml of 0.5 M perchloric acid. The eluate was diluted with 10 ml of doubly distilled water before the determination of single metals by AAS.

Trace amounts of calcium, magnesium, iron, copper and lead were also determined in a single sample of Suprapur sodium carbonate after pre-concentration of trace metals on the column packed with 1 g of the sorbent. A volume of 200 ml of 5% *m/V* sodium carbonate solution was passed through the column and the retained metals were eluted with 5 ml of 0.5 M perchloric acid. A 5-ml volume of doubly distilled water was added to the eluate before the determination of the single metals. Silica gel coated with a mixture of Aliquat 336 and Eriochrome Black T was also applied to the concentration of trace amounts of calcium, magnesium, iron, copper and lead from single samples of distilled, doubly distilled and de-

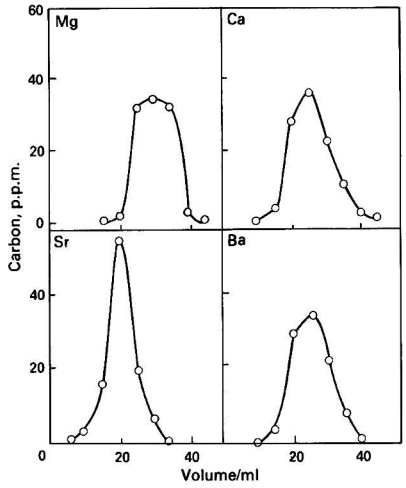

Fig. 1. Elution curves for alkaline earth metals. Column length, 165 mm; eluent, 0.0075 M $HClO_4$; mean flow-rate, 1 ml min^{-1}

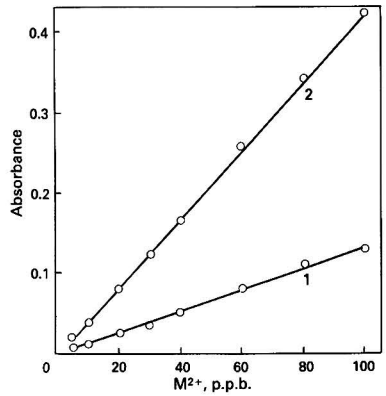

Fig. 2. Calibration graphs of the atomic-absorption spectrometric determination of (1) calcium and (2) magnesium after their pre-concentration on small columns containing 0.3 g of silanised silica coated with Aliquat 336 and Eriochrome Black T

ionised water. A 200-ml volume of the sample water was passed through the column, which was subsequently washed with 10 ml of doubly distilled water from a quartz apparatus and the retained metals were eluted with 5 ml of 0.5 M perchloric acid; the eluate was diluted with 10 ml of doubly distilled water. The results of the determination of retained metals in some salts and in water samples are given in Table 3.

The proposed method for the concentration of trace amounts of metals before their determination in chemical reagents was verified with the use of the standard additions method. After passing 50 ml of 1% metal salt salt solution through the column packed with 0.3 g of the sorbent, 200 ml of a standard solution of calcium, magnesium, iron(III), copper and lead salts (containing 10 μg of each metal ion) were passed through the column. A 5-ml volume of 0.5 M perchloric acid was used for elution and the eluate was then diluted with 5 ml of doubly distilled water before determination of metals by AAS (Table 4).

Table 3. Determination of Ca, Mg, Fe, Cu and Pb in some sodium, potassium and ammonium salts and in water after pre-concentration of the metals on small columns packed with 0.3 g of sorbent A

	Concentration of impurities, % × 10^{-4}				
Sample	Ca	Mg	Fe	Cu	Pb
Potassium chloride, AR*	10	9	5.5	1	1
Potassium nitrate, AR	9	8	6	9	1.1
Sodium chloride, pure	12	11	9	1.2	1.4
Sodium nitrate, AR	8	9	8	1	4
Ammonium chloride, AR	8	6	7	8	1.1
Ammonium nitrate, AR	6	7	6	8	1
Sodium carbonate, Suprapur	0.3	1	<0.1	0.15	<0.02
Distilled water	0.1	0.38	0.04	0.005	0.04
Doubly distilled water	0.06	0.27	0.02	—†	0.02
De-ionised water	0.14	0.22	0.008	—†	0.01

* Analytical-reagent grade.
† Not detectable by AAS with flame atomisation.

Table 4. Determination of Ca, Mg, Fe, Cu and Pb in some salts after pre-concentration of retained metals and passage through the column of a standard solution of the metals (containing 10 μg of each metal ion). The amounts of metals in the eluate without addition of standard metal salt solution are given in parentheses

	Amount of impurity in eluate/μg				
Salt	Ca	Mg	Fe	Cu	Pb
Potassium nitrate, AR*	14.5 (4.5)	14.0 (4.0)	13.0 (3.0)	10.4 (0.45)	10.5 (0.55)
Sodium chloride, pure	16.0 (6.0)	15.5 (5.5)	14.4 (4.5)	10.6 (0.6)	10.8 (0.7)
Ammonium nitrate, AR	14.0 (4.0)	13.2 (3.0)	13.6 (3.5)	10.4 (0.4)	10.5 (0.55)

* Analytical-reagent grade.

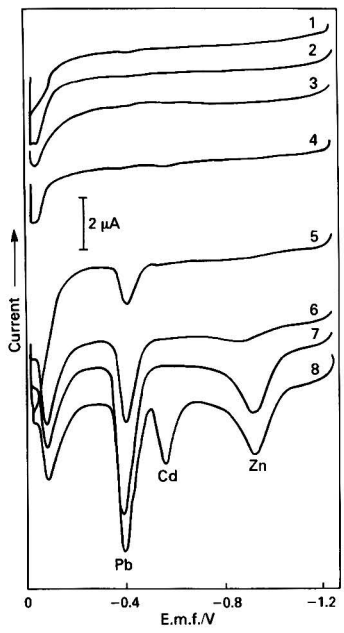

Fig. 3. Anodic-stripping voltammetric determination of Pb, Cd and Zn in eluates after passing 5% *m/V* solutions of purified salts (to which the mixture of standard solutions of Cu, Pb, Cd and Zn was added) through small columns packed with 1 g of the sorbent D. Time of electrolysis; 5 min for the purified salts and 2 min for the purified potassium chloride with addition of a standard solution of lead and cadmium. Curves: 1, ammonium chloride; 2, sodium chloride; 3, sodium acetate; 4, potassium chloride; 5, potassium chloride diluted two-fold with distilled water and polluted with trace amounts of lead; 6, sample 5 + Pb^{2+} (5 × 10^{-8} M); 7, sample 5 + Pb^{2+} (10^{-7} M), accidentally polluted with zinc; and 8, sample 7 + Cd^{2+} (5 × 10^{-8} M)

Purification of Sodium, Potassium and Ammonium Salts

As bivalent and trivalent metal ions are very strongly retained on silica treated with a mixture of Aliquat 336 and Eriochrome Black T from moderately acidic and alkaline aqueous solutions, the proposed sorbent can be used for additional purification of analytical-reagent grade sodium, potassium and ammonium salts. The following salts were purified: sodium chloride, sodium acetate, ammonium chloride and potassium chloride. A 10-ml volume of a 5% *m/V* solution of the purified salt was passed through the small column packed with 1 g of sorbent D and the eluate was analysed by AAS or ASV. To several samples of purified salt solutions, a mixture of standard solutions of magnesium, calcium, iron, cobalt, nickel, magnesium, zinc, cadmium, copper and lead salts (containing 100 μg of each metal ion) was added. It was found that none of the metals present as impurities in the sample salts can be determined by AAS with flame atomisation. Taking into account the sensitivity of this method, the concentrations of magnesium and cadmium in the eluate must have been lower than 10^{-6}%.

Lead, cadmium and zinc were additionally determined in eluates from the column by ASV. Fig. 3 shows typical voltammograms for solutions of purified salts to which a mixture of standard solutions of lead (4.8 × 10^{-5} M), cadmium (8.9 × 10^{-5} M), zinc (1.5 × 10^{-4} M) and copper (1.6 × 10^{-4} M) had been added. The results indicated that the concentrations of lead, cadmium and zinc in the eluates from the column were lower than 10^{-9} M, which confirms the usefulness of silica gel treated with a mixture of Aliquat 336 and Eriochrome Black T for removing, very effectively, trace amounts of bivalent and trivalent metal ions from high-purity reagents.

Conclusion

Silica gel coated with a mixture of Aliquat 336 and Eriochrome Black T can be used for the pre-concentration of trace amounts of bivalent and trivalent metal ions present as

impurities in alkali metal salts, in ammonium salts and in water before the determination of these metals by conventional analytical methods. The retained metals can be eluted from the column with dilute solutions of mineral acids, which simultaneously leads to the regeneration of the sorbent.

The proposed sorbent can also be used for additional purification of analytical-reagent grade sodium, potassium and ammonium salts. For instance, it was found that the concentrations of lead, cadmium and zinc were decreased by at least five orders of magnitude after passing the solutions of purified salts through the column packed with the sorbent. As bivalent and trivalent metal ions are retained even from moderately acidic aqueous solutions, it is expected that the sorbent will be useful for the purification of aqueous solutions of weak acids and of very dilute solutions (lower than 10^{-3} M) of strong mineral acids.

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Sorption of Uranium by Cellulose Derivatives

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The sorption behaviour of uranium was investigated by batch and column methods using four cellulose derivatives, two having an amino group and two having an amino group plus a dithiocarboxylate group. All four derivatives showed maximum uranium sorption from pure water in the neutral region or from artificial sea water in the acidic region. In the batch method, quantitative removal of 100 μg of uranium from 50 ml of water at pH 5.0 was achieved by equilibration with 50 mg of any of the four sorbents. The percentage removal under these conditions decreased gradually with increasing concentration of uranium above 100 μg per 50 ml. A similar high percentage removal of uranium was achieved using artificial sea water. In the column method, quantitative removal of 5 μg of uranium from 1 l of water at pH 6.7 was achieved with 200 mg of either of the two sorbents. Similar quantitative removal of uranium was achieved from 1 l of artificial sea water at pH 5.4 with 500 mg of either of the two sorbents. Several 1- and 10-l portions of natural sea water, adjusted to pH 5.0, were passed through the column, which was packed with a mixture of 500 mg of either of the two sorbents and 1 g of cellulose powder. The results indicated that uranium was quantitatively removed from the 1-l portions, while approximately 50% was removed from the 10-l portions.

Keywords: *Uranium sorption; dithiocarboxylate cellulose; amine cellulose; Arsenazo III; spectrophotometry*

Problems have been encountered in the recovery of uranium from sea water, because of the concentration (as low as 3 p.p.b.) and the various species that coexist. Sorbents used to remove uranium from sea water have included metatitanic acid,[1] chelating agents such as hexaketone,[2] amideoxime[3] and others.[4,5] A method employing coprecipitation on magnesium hydroxide has also been reported,[6] and it has been reported that dithiocarboxylate cellulose is an excellent sorbent for many metal ions.[7–9] In this study, the sorption behaviour of uranium from artificial sea water or pure water, by cellulose derivatives, was investigated. In addition, the removal of uranium from natural sea water was also examined.

Experimental

Reagents

All reagents were of analytical-reagent grade.

Uranium stock standard solution, 1000 p.p.m. Dissolve 1.68 g of $UO_2(CH_3CO_2)_2.2H_2O$ in 5% acetic acid and dilute to 1 l with water.

Arsenazo III solution, 0.2%. Dissolve 0.2 g of Arsenazo III in 100 ml of water with stirring overnight.

Ammonium acetate buffer solution, 10%, *pH* 7.2. Dissolve 10 g of ammonium acetate in water and dilute to 100 ml.

Ammonium chloride buffer solution, 10%, *pH* 5.0. Dissolve 10 g of ammonium chloride in water and dilute to 100 ml.

Perchloric acid, 60%.

Hydrochloric acid, 1 N.

Ammonia solution, 1 N.

Cellulose powder, 100–200 *mesh*.

Table 1. Composition of artificial sea water. The over-all chloride concentration was 19.00 g l^{-1}

Salt	Concentration/ g l^{-1}	Salt	Concentration/ g l^{-1}
NaCl	23.476	$NaHCO_3$	0.192
$MgCl_2$	4.981	KBr	0.096
Na_2SO_4	3.917	H_3BO_3	0.026
$CaCl_2$	1.102	$SrCl_2$	0.024
KCl	0.0664	NaF	0.003

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Artificial sea water. Dissolve the components shown in Table 1 in doubly distilled water and dilute to 1 l.

Sorbents. n-Butylamino cellulose (BUA), dithiocarboxylate cellulose (BUD) derived from BUA, piperazine cellulose (PIA) and dithiocarboxylate cellulose (PID) derived from PIA were prepared according to the procedures described previously.[7,8]

Apparatus

A Shimazu UV-200 double-beam spectrophotometer, a Trapelo PDS-504 dry stripper low-temperature plasma asher and a Hitachi-Horiba Type F-5_{ss} pH meter were used.

Procedure

Batch method

A 50-ml volume of pure water or artificial sea water was placed in a beaker, certain volumes of the standard solutions that were diluted to 10 or 100 p.p.m. from suitable aliquots of the uranium stock standard solution were added and 2 ml of 10% ammonium acetate solution or 2 ml of 10% ammonium chloride were added as required. After the addition of 50 or 500 mg of the sorbent to this solution, stirring was commenced and the pH was adjusted to the specified value with hydrochloric acid and ammonia solution. Stirring was continued for 30 min. The sorbent was then separated with a membrane filter (Millipore Filter RAWP-047) and the filtrate was diluted to 100 ml with water. Uranium in the filtrate was analysed spectrophotometrically using Arsenazo III as a colour-producing reagent.[10]

A 10-ml aliquot of the filtrate was placed in a 50-ml calibrated flask. A 26-ml volume of 60% perchloric acid and 5-ml of water were added, and the mixture was shaken thoroughly and allowed to cool to room temperature. A 4-ml volume of 0.2% Arsenazo III solution was added and the solution was diluted to 50 ml with water. The absorbance was then measured in 1-cm cells at 655 nm against a solution that had been prepared in a similar manner but without the addition of uranium. When the concentration of uranium in the sample solution was very low, cylindrical glass cells of 25 mm diameter and 100 mm long were used.

Column method

A mixture of 200 mg of sorbent and 800 mg of powdered cellulose or a mixture of 500 mg of sorbent and 1 g of powdered cellulose were packed in a column with an inside

diameter of 10 mm, through which 1 l of the sample solution containing 5–100 μg of uranium and adjusted to pH 6.7 or 7.4 was passed at a rate of 200 ml h^{-1} without suction. Next, the sorbent was separated with a membrane filter and washed thoroughly with water. It was placed in a quartz boat to be dried at 110 °C and ashed in a low-temperature plasma asher for approximately 12 h under the following conditions: oxygen flow-rate, 40 ml min^{-1}; vacuum pressure, 1 Torr; and high frequency power, 100 W. The ash remaining in the quartz boat was dissolved in 10 ml of 60% perchloric acid and placed in a 50-ml calibrated flask. After 16 ml of 60% perchloric acid and 15 ml of water had been added, the solution was shaken thoroughly and cooled to room temperature. A 4-ml volume of 0.2% Arsenazo III solution was added and diluted to 50 ml with water. The solution was measured as described under *Batch method*.

Results and Discussion

Dependence on pH

The effect of pH on the sorption of uranium by the four sorbents in pure water was examined by the batch method. The amount of the sorbent was 50 mg and the amount of uranium added was 100 μg. As shown in Fig. 1, PIA and PID sorb well at pH values of 5–9 and 4–7, respectively. BUA and BUD both sorb well at pH values of 5–8.

Sorption experiments were carried out with artificial sea water using 500 mg of sorbent and 100 μg of uranium. As shown in Fig. 2, both PIA and PID showed good sorption over the acidic to neutral range, and BUA and BUD showed maximum sorption over a pH range of 4–6. The difference in sorption behaviour between pure water and artificial sea water is probably due to the carbonate ion contained in the latter forming a stable complex with uranium, the effect becoming greater as the pH rises.

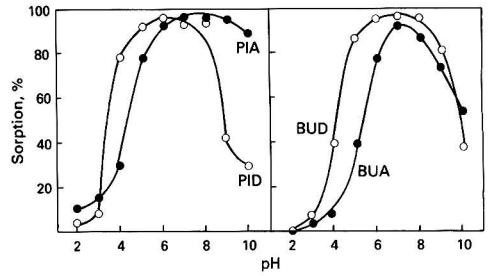

Fig. 1. Effect of pH on the sorption of uranium from pure water. Uranium, 100 μg; water, 50 ml; sorbent, 50 mg; and equilibration time, 30 min

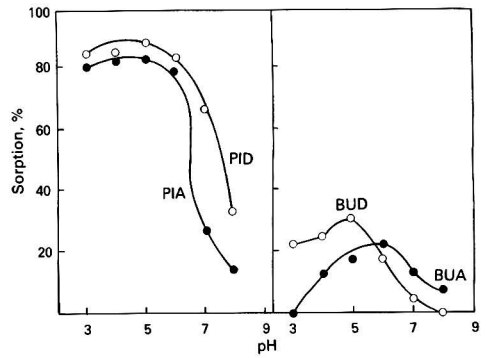

Fig. 2. Effect of pH on the sorption of uranium from artificial sea water. Uranium, 100 μg; water, 50 ml; sorbent, 500 mg; and equilibration time, 30 min

Relationship Between Amount of Uranium and Amount Sorbed

The effect of agitation time on sorption in the batch method was determined using 50 mg of PIA or PID and 100 μg of uranium in pure water. Sorption was determined at pH 6 for agitation times of 5 min, 15 min, 30 min, 2 h, 5 h and 24 h. As shown in Fig. 3, the sorption is constant when the agitation time exceeds 15 min. The agitation time was, therefore, fixed at 30 min in subsequent tests.

Variation of sorption with concentration was studied using between 50 and 100 μg of uranium in 50 ml of pure water at pH 5 and with 50 mg of sorbent. The results are shown in Fig. 4. A 50-μg mass of uranium is sorbed 100% on PID but the extent of sorption falls with increasing amount of uranium; the sorption was 56% for 1000 μg. The extent of sorption by PIA is slightly lower than that by PID.

Similar tests were carried out with artificial sea water using between 100 and 2000 μg of uranium, a pH of 5.0 and 500 mg of sorbent. The results are shown in Fig. 5. The sorption trends are almost the same as those observed for pure water. Sorption from solutions containing 2000 μg of uranium are 75 and 60% by PID and PIA, respectively. The amounts of uranium sorbed from artificial sea water per unit mass of sorbent are about half of the amounts sorbed from pure water. This effect is probably due to the presence of carbonate ion in artificial sea water.

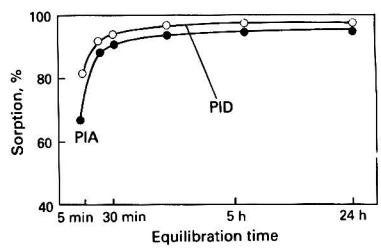

Fig. 3. Effect of equilibration time on the sorption of uranium from pure water. Uranium, 100 μg; water, 50 ml; sorbent, 50 ml; and pH, 6.0

Recovery by the Column Method

Solutions containing 5, 25 and 100 μg of uranium in 1 l of pure water at pH 6.7 were passed through columns packed with a mixture of 200 mg of sorbent and 800 mg of powdered cellulose, and the extent of removal of uranium was measured.

The results listed in Table 2 reveal that removal by BUD or PID is almost 100% for 5 μg of uranium, nearly 90% for 25 μg and approximately 50% for 100 μg. Similar tests were carried out with 5 or 10 μg of uranium in 1 l of artificial sea water with the pH adjusted to 7.4. Table 3 shows that quantitative removal was not achieved for any system. When the amounts of sorbent and powdered cellulose were increased to 500 mg and 1 g, respectively, it was found that 5 g of uranium were quantitatively removed from 1 l of artificial sea water adjusted to pH 5.4 by a column containing either PIA or PID.

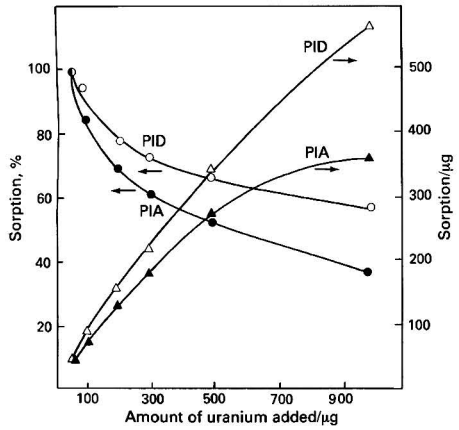

Fig. 4. Comparison of sorbents for the sorption of uranium from pure water. Water, 50 ml; sorbent, 50 mg; and equilibration time, 30 min

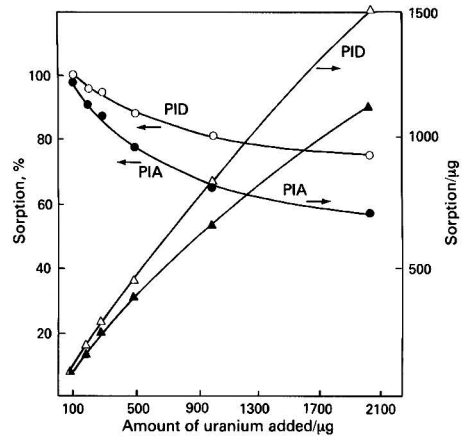

Fig. 5. Comparison of sorbents for the sorption of uranium from artificial sea water. Sea water, 50 ml; sorbent, 500 mg; and equilibration time, 30 min

The possibility of loss of uranium by volatilisation during low-temperature plasma ashing was examined by adding 100 μg of uranium to 1 g of sorbent - cellulose mixture and following the usual ashing procedure. No loss of uranium was observed under these conditions.

Sorption of Uranium from Natural Sea Water

Sea water from near Kobe City was filtered through a membrane filter immediately after sampling, and the pH of the filtrate was adjusted to 5.0. Several 1- and 10-l portions of the sample were passed through columns packed with mixtures of 500 mg of PIA or PID and 1 g of cellulose powder. After passage, the packing was removed, dried and treated in the low-temperature plasma asher. Uranium was determined using the procedure already described. The results, shown in Table 4, indicate that uranium was quantitatively removed from the 1-l portions, while approximately 50% of the uranium was removed from the 10-l portions.

Table 2. Results for the removal of uranium by the column method from 1 l of pure water. Each column contained a mixture of 200 mg of sorbent and 800 mg of cellulose at a pH of 6.7

Sorbent	Amount added/μg	Amount found/μg	Recovery, %
BUD	5.0	4.8	96
PID	5.0	5.0	100
BUD	25.0	23	92
PID	25.0	22	88
BUD	100	47	47
PID	100	45	45

Table 3. Results for the removal of uranium by the column method from 1 l of artificial sea water. Each column contained a mixture of 200 mg of sorbent and 800 mg of cellulose at a pH of 7.4

Sorbent	Amount added/μg	Amount found/μg	Recovery, %
BUA	5.0	3.1	62
PIA	5.0	2.8	56
BUD	5.0	1.8	36
PID	5.0	3.7	74
BUA	10.0	2.7	27
PIA	10.0	2.8	28
BUD	10.0	3.1	31
PID	10.0	3.4	34

Table 4. Amount of uranium sorbed by the column from natural sea water. Natural sea water was sampled from the coast of Kobe City and pre-adjusted to a pH of 5.0

Sorbent	Sample volume/l	Uranium found/μg
PIA	1	3.2
PID	1	3.1
PIA	10	16.9
PID	10	15.1

Conclusion

The sorption behaviour of uranium from aqueous solution by four derivatives of cellulose having an amino group, with or without a dithiocarboxylate group, was investigated. The extent of sorption depends on the pH of the solution. Each sorbent shows maximum sorption in the neutral region for pure water and in the acidic region for artificial sea water. Using the batch method, the proportion of sorbed uranium gradually decreased as the amount of uranium was increased. Using the column method, 5 μg of uranium were quantitatively removed from 1 l of artificial sea water at pH 5.0 by 500 mg of PID and 15–17 μg of uranium were removed from 10 l of sea water.

These results indicate that these sorbents are effective chelating agents for the collection of uranium from natural waters. The procedure is simple and leads to the concentration of uranium together with other metallic elements that are sorbed simultaneously. Subsequent elution would be necessary in order to separate uranium in a pure form from sea water.

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Silver Chloride Pre-treatment for the Direct Potentiometric Determination of Chloride in Stream Waters Using a Solid-state Chloride Ion-selective Electrode

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An effective pre-treatment for the direct potentiometric determination of chloride using a solid-state chloride ion-selective electrode is proposed, which is based on the addition of a freshly prepared suspension of silver chloride to a stream water sample. Bromide in the sample can be effectively removed by solubility exchange with chloride from the silver chloride suspension, thus removing the interference caused by silver bromide being formed on the electrode surface. Interference from iodide and sulphide can also be suppressed, although the degree of interference from these ions in low concentrations is much smaller than would be expected from the solubility products of their silver salts.

The 1–17% positive errors observed in the analysis of ten stream waters containing 6.67–40.0 mg l^{-1} of chloride have been decreased to below 5% by the proposed pre-treatment.

Keywords: *Chloride determination; chloride ion-selective electrode; direct potentiometry; stream water analysis*

The determination of chloride in natural waters is important because it is contained in domestic effluents or industrial sewages and its concentration can be regarded as an indicator of water pollution.

Potentiometric titration with silver nitrate is a precise method for the determination of chloride,[1] but because direct potentiometry using a chloride ion-selective electrode is both rapid and simple it is a more suitable technique for the analysis of stream waters because the concentration of chloride varies greatly from one stream to another and all samples should be analysed collectively.

Many papers have been published on the direct potentiometric determination of chloride in natural waters.[2–4] Because it is known that a chloride ion-selective electrode based on a solid-state silver chloride - silver sulphide electrode, suffers from interference from bromide, sulphide etc., some pre-treatment is usually required in water analysis. For example, Selmer-Olsen and Øien[2] brought the pH of the sample solution to 3 by the addition of nitric acid. van Oort *et al.*[3] pointed out the possibility of positive interference from organic species in polluted water, which could be effectively suppressed by the addition of acetic acid. Takamatsu *et al.*[4] removed the interference from sulphide by the addition of lead(II). Hori *et al.*[5] recommended the addition of nickel nitrate and potassium permanganate to remove the interference from sulphide, cyanide and iodide in a waste water.

In stream water analysis, we have experienced some interferences even in an unpolluted water. The concentration levels of bromide, iodide and sulphide in stream waters are acknowledged to be around 0.03, <0.01 and <0.01 mg l^{-1}, *respectively, while the concentration level of chloride lies between 5 and 50 mg* l^{-1}. *If the selectivity coefficients based* on solubility data of these ions remain unchanged even at such low concentrations, it is still possible that these ions will interfere.

In this work, we have evaluated the interference from bromide, iodide and sulphide and concluded that bromide was the most probable interferent among these inorganic ions, although there remains the possibility of interference from some organic species, especially in polluted stream waters. It was confirmed that the addition of a freshly prepared silver chloride suspension was an effective pre-treatment to remove the interference from bromide, sulphide and iodide and other species that might interfere.

Experimental

Apparatus

An Orion 94-17B solid-state chloride ion-selective electrode and an Orion 90-02 double-junction type reference electrode were used. The outer filling solution of the reference electrode was 5 M sodium nitrate solution. The electrode potentials at room temperature (26 ± 1 °C) were measured to an accuracy of 0.1 mV with an Orion Model 701A Ionalyzer and recorded with a Rikadenki Model R-10 pen recorder. The potential value was transmitted every 0.5–0.7 s to an NEC PC8801 MkII microcomputer through a suitable interface and the mean value over 10 s was calculated. The difference between the two mean potential values over a period of 1 min was monitored successively and an equilibrium potential was defined when the potential difference decreased to 0.1 mV min^{-1}. Although such a definition seems to be arbitrary, it was adequate for the quantitative evaluation of interference because the potential continued to drop very slowly to a more negative value owing to the lingering interference.

Reagents

Analytical-reagent grade potassium chloride, dried at 110 °C for 2 h, was used to prepare a standard solution of chloride (1000 mg l^{-1}). Sodium sulphide was dried under reduced pressure at room temperature. Other reagents were used without further purification.

Procedure

The titrimetric procedure with silver nitrate has been described previously.[1] The direct potentiometric procedure was as follows. Add 1 ml of 5 M sodium nitrate solution, as an ionic strength adjuster, and 1 ml of silver chloride solution to a 50-ml volume of sample solution. After allowing the mixture to stand for 1 h, which is sufficient for the effective removal of interferents, measure the electrode potential.

The 3.13×10^{-3} M silver chloride solution, containing 500 mg l^{-1} of Tween 80 (polyoxyethylene sorbitan monooleate) to make a stable colloid, was prepared by mixing solutions of silver nitrate and potassium chloride. Standard solutions for calibration containing 5, 10, 20 and 50 mg l^{-1} of chloride were

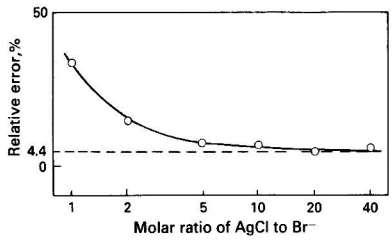

Fig. 1. Effect of silver chloride concentration on relative error. The sample was a mixture of 10 mg l^{-1} chloride and 1 mg l^{-1} bromide solutions. The ionic strength was adjusted to 0.1 using 5 M sodium nitrate solution

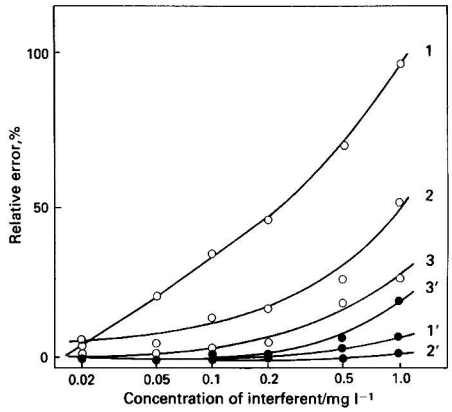

Fig. 2. Effect of silver chloride on the interference from (1) bromide, (2) iodide and (3) sulphide. Graphs 1–3, without silver chloride; and 1′–3′, with 1 ml of 3.13×10^{-3} M silver chloride solution added to 50 ml of a 10 mg l^{-1} chloride solution. The ionic strength was adjusted to 0.1 using 5 M sodium nitrate solution

prepared by the serial dilution of potassium chloride (1000 mg l^{-1} of chloride) and used after the same pre-treatment as that of sample. The interferences from bromide, iodide and sulphide were evaluated in a mixture of 10 mg l^{-1} chloride and each of the potassium or sodium salts. The apparent chloride concentration was determined from the calibration graph of chloride and the relative error was used as a quantitative measure of interference.

The effect of pH on the interference of bromide was observed in a solution of 10 mg l^{-1} of chloride and 0.1 mg l^{-1} or 1 mg l^{-1} of bromide. The pH of the solution was controlled with nitric acid (pH 2), acetate buffer (pH 3.8) and phosphate buffer (pH 6.8); an unbuffered solution (pH 5.7) was also measured. The ionic strength was adjusted to 0.1 by adding a suitable amount of sodium nitrate.

Results and Discussion

Electrode Response

The electrode showed a linear response over the concentration range 5–100 mg l^{-1} or greater with a slope of 58 ± 1 mV decade^{-1}. The lower limit is adequate for stream water analysis because the concentration level of chloride is usually above 5 mg l^{-1}. The equilibrium potential was obtained within 2 min and was reproducible to an accuracy of 0.5 mV.

Measurement Conditions

From a practical point of view, the upper limit of interferent concentration was defined as 1 mg l^{-1} for 10 mg l^{-1} of chloride because, for bromide, the complete ion exchange gives a 4.4% positive error, which corresponds to an error of 1 mV in the potential reading for the direct potentiometry of monovalent species. The effect of the concentration of silver chloride was examined as shown in Fig. 1. The error approaches a limit of 4.4% corresponding to an increase in the molar ratio of silver chloride to bromide, which supports the ion-exchange mechanism.

In considering the actual concentration level of bromide, a molar ratio of five was adopted. Silver chloride did not show an appreciable effect on the electrode response even for a molar ratio of 40. The effect of the standing time was also examined with a mixture of 0.1 mg l^{-1} of bromide and 10 mg l^{-1} of chloride. A standing time of 1 h was sufficient for obtaining the minimum error of about 1%.

Interference from Bromide, Iodide and Sulphide

The interference from these ions on the determination of 10 mg l^{-1} of chloride is shown in Fig. 2. The relative error was used as a measure of interference. Unexpectedly, the interfering effect of bromide was greatest. The selectivity coefficient of bromide was between 10 and 100; the reproducibility of the interference from iodide and sulphide was too poor to obtain a reliable selectivity coefficient. In any event, the selectivity coefficients of these ions were much smaller than the values expected from the solubility products of their silver salts.

Rhodes and Buck[6] examined the bromide interference on rotating anodised silver - silver chloride electrodes and reported that the selectivity coefficient varied from 10 to 450 depending on the degree of surface coverage and measuring time. Klasens and Goossen[7] reported that the selectivity coefficient of iodide with a Philips chloride electrode ranged from 1.4 to 14 and explained this as a result of silver iodide deposition on the membrane. Narasimhan and Visalakshi[8] pointed out that the degree of interference from these ions cannot be predicted from their solubility products alone.

Because the concentrations of iodide and sulphide in stream waters are usually both below 0.01 mg l^{-1}, it was concluded from the results shown in Fig. 2 that interference from these ions was negligible. However, the interference from bromide should be appreciable, as the concentration of bromide in stream waters is presumed to be about 0.03 mg l^{-1}.[9]

When the concentration of bromide was 0.05 mg l^{-1}, the potential changed very slowly to a more negative value. The equilibrium potential defined previously was found to be dependent on the measuring conditions, such as the speed of stirring.

Interference from low concentrations of bromide and iodide can be suppressed by the addition of silver chloride. On consideration of the solubility products of silver bromide and silver chloride, the ion-exchange mechanism may not be the predominant factor when the concentration of bromide is lower than 0.07 mg l^{-1}. Nevertheless, this pre-treatment was still effective even below this concentration level. In addition to the ion-exchange mechanism, the specific adsorption of bromide on to the silver chloride precipitate may also have an effect at low concentrations.

Table 1. Effect of pH on the interference of bromide. Concentration of chloride, 10 mg l^{-1}; ionic strength, 0.1

	0.1 mg l^{-1} of Br^-		1 mg l^{-1} of Br^-	
pH	Relative error, %	R.s.d.* ($n = 5$), %	Relative error, %	R.s.d. ($n = 5$), %
2.0	27.0	4.1	79.2	10.0
3.8	26.8	6.5	74.8	11.7
5.7	34.8	4.7	97.4	5.6
6.8	32.2	9.0	96.6	15.8

* R.s.d., relative standard deviation.

Table 2. Results of the direct potentiometric determination of chloride in stream waters

No.	Concentration of Cl^- by titration/ mg l^{-1}	Concentration of Cl^- by direct potentiometry* Without AgCl/ mg l^{-1}	Error, %	With AgCl/ mg l^{-1}	Error, %
1	6.67	7.56	13	6.68	0
2	10.1	11.8	17	10.0	−1
3	11.5	13.3	16	11.4	−1
4	14.4	16.2	13	14.1	−2
5	16.3	18.5	13	16.1	−1
6	17.2	18.7	9	17.1	−1
7	18.3	19.4	6	18.6	2
8	23.7	26.4	11	25.0	5
9	26.2	28.5	9	26.1	0
10	40.0	40.5	1	38.9	−3

* A 1-ml volume of 5 M sodium nitrate solution and a 1-ml volume of 3.13×10^{-3} M silver chloride solution were added to 50 ml of sample solution.

Table 3. Results of the precision and recovery test

Stream	Concentration of Cl^- by titration/mg l^{-1}	Concentration of Cl^- by direct potentiometry* Without AgCl: Found/ mg l^{-1}	S.d./ mg l^{-1}	Error, %	With AgCl: Taken/ mg l^{-1}	Found/ mg l^{-1}	S.d./ mg l^{-1}	Error, %	Recovery, %
Tara	16.0	17.6	—	10	—	16.3	0.63	2	
					5	21.2	0.19		98
					10	26.7	0.14		104
Senjyo . .	8.04	8.65	0.21	8	—	8.00	0.068	0	
					5	13.2	0.084		104

* The results are mean values of five independent measurements.

An error of 21% is expected to be occur for 1 mg l^{-1} of sulphide when complete ion exchange occurred. The result obtained supported this expectation. Therefore, the effective suppression concentration of sulphide was below 0.2 mg l^{-1}; at higher concentrations an oxidative pre-treatment will be more effective.[5]

Effect of pH

The effect of pH on the interference from bromide was examined and the results are listed in Table 1. Generally, the pH had no significant effect although a slight decrease in the relative error at both concentrations was found from pH 5.7 to 3.8. Although the exact reason for this pH dependence is not clear, it was observed that complete removal of the interference from bromide was impossible by acidification of the sample alone.

Effect of Silver Chloride Pre-treatment

Ten stream waters from Shiga Prefecture were analysed with and without silver chloride pre-treatment. The results were compared with those obtained using titration with silver nitrate and are shown in Table 2. In considering the reproducibility of the potential measurements, an error of ±2% is allowable. Except for one measurement, the agreement between the values obtained using the two methods was excellent irrespective of the wide concentration range of chloride. Both the accuracy and the potential response were improved by the proposed pre-treatment. The response time was decreased to 1 min for most samples.

Precision and Recovery Test

Two stream waters, Tara and Senjyo, were chosen for the precision and recovery test. The former is polluted by domestic effluents and the latter is not polluted (by domestic sewage or industrial waste). The results are shown in Table 3. Although the recovery was satisfactory in both samples, a small error still remained after the silver chloride pre-treatment of Tara water. The long response time (about 4 min) also meant that interference still remained. This result may be attributable to the presence of various species that can react with the silver ion, including organic species. Increasing the concentration of silver chloride was effective for the analysis of such a polluted water.

Conclusion

The most important feature of the pre-treatment is that it can effectively suppress interference from bromide, which cannot be removed by other pre-treatments such as acidification or oxidation. As the interference on a silver chloride electrode occurs when an ion-exchange reaction occurs between chloride in the membrane and an interferent in the solution, this pre-treatment is effective for all interferents that can react with silver chloride, in principle, if the chloride released by the ion-exchange reaction is insignificant compared with that present initially. This pre-treatment can be used not only for stream water analysis but for any water sample in which a significant error is obtained from the presence of small amounts of interferents. However, the exact mechanism is still ambiguous in the concentration range below the solubility product.

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Construction of Ion-selective Electrodes for Chlorpromazine, Amitriptyline, Propantheline and Meperidine: Analytical Study and Application to Pharmaceutical Analysis

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Liquid-membrane ion-selective electrodes that respond to the cationic forms of chlorpromazine, amitriptyline and meperidine and to the quaternary ammonium compound propantheline are described. The liquid ion exchangers used were the salts of chlorpromazine or amitriptyline with eosin and tetraphenylborate in *p*-nitrocumene, propantheline with tetraphenylborate in 2-nitrotoluene and meperidine with tetrakis(*m*-chlorophenyl)borate in *p*-nitrocumene. The electrodes exhibited near-Nernstian response in the range 6×10^{-3}–4×10^{-5} M, working pH range 1–6 (chlorpromazine); 6×10^{-3}–6×10^{-5} M, pH 1–6 (amitriptyline); 6×10^{-3}–3×10^{-6} M, pH 1–9 (propantheline); and 10^{-2}–7×10^{-6} M, pH 1–7 (meperidine). The interferences from the alkali and alkaline earth metals were negligible but cationic molecules of similar structure interfered strongly. The electrodes were applied successfully to the assay of active compounds in pharmaceutical preparations. The major advantages of the proposed methods are their simplicity and speed.

Keywords: *Ion-selective electrodes; chlorpromazine; amitriptyline; propantheline; meperidine*

Ion-selective electrodes (ISEs) have found many successful applications in pharmaceutical analysis,[1–7] mainly because of their low cost, ease of use and maintenance and the simplicity and speed of the assay procedures. It is usually possible to develop procedures for the determination of drugs in pharmaceutical preparations that need only a pre-dilution step with a suitable buffer (*e.g.*, injection preparations) or dissolution of tablets in the measuring solvent. Turbidity due to the tablet matrix is not usually a problem, so that even the filtration step can be avoided.

The most important limitation of ISEs is their poor selectivity, especially for compounds of similar structure. With multi-component drugs, it is sometimes possible to measure specifically a single compound by optimisation of the analytical parameters.

In this paper, the construction of four liquid-membrane ISEs that respond to the cationic forms of the drugs chlorpromazine, amitriptyline, propantheline and meperidine is described. Chlorpromazine is the most widely used major tranquilliser, amitriptyline is a major tricyclic antidepressant, propantheline is an anticholinergic drug used extensively for the treatment of peptic ulcers and meperidine is a potent analgesic. An analytical study of the electrodes showed that they exhibit satisfactory sensitivity and selectivity and a broad working pH range. Titrimetric and direct potentiometric procedures have been developed for the rapid and simple assay of the active compounds present in various pharmaceutical formulations.

Experimental

Apparatus

All electrodes were used with a single-junction Ag - AgCl electrode as the reference (Orion Model 90-01-00). Potential readings were obtained with a Corning Research pH/mV meter (Model 12), and recorded simultaneously on a strip-chart recorder. All titrations were carried out with a Radiometer system consisting of a multi-speed constant-rate burette (Model ABU 12) and a recorder (REC 61) equipped with an REA 112 high-sensitivity unit. All measurements were carried out at room temperature with constant magnetic stirring.

Reagents

All solutions were prepared in de-ionised, distilled water from analytical-reagent grade materials. Stock solutions (0.1000 M) of chlorpromazine hydrochloride, amitriptyline hydrochloride, propantheline bromide and meperidine hydrochloride were prepared in water. The pure substances used were of the highest quality available, and were gifts from various pharmaceutical companies. All solutions were stored at room temperature except for chlorpromazine, which was stored in amber bottles at 4 °C to avoid photochemical oxidation.[8] Pharmaceutical preparations were obtained from local pharmacies.

Liquid Ion Exchangers

The cationic forms of chlorpromazine, amitriptyline, propantheline and meperidine were precipitated from aqueous solutions with bulky anions. The resulting insoluble compounds were extracted into organic solvents and the extracts were dried with anhydrous sodium sulphate and used to fill the electrodes. The best liquid ion exchanger for each compound was prepared as described below. Less satisfactory liquid ion exchangers were prepared in a similar way and are described later.

Chlorpromazine. Chlorpromazine was precipitated by mixing 1 ml of sodium tetraphenylborate solution (5×10^{-2} M), 1 ml of sodium eosin solution (5×10^{-2} M) and 1.5 ml of chlorpromazine hydrochloride solution (0.10 M). The precipitate was extracted with 5 ml of *p*-nitrocumene (4-isopropylnitrobenzene) (Fluka) and the organic phase was washed three times with water. The organic solution was dried by adding 0.5 g of sodium sulphate, which was then removed by centrifugation.

Amitriptyline. The same procedure as for chlorpromazine was used, but amitriptyline hydrochloride was used instead of chlorpromazine hydrochloride.

Propantheline. Propantheline was precipitated by mixing 2 ml of sodium tetraphenylborate solution (5×10^{-2} M) and 2 ml of propantheline bromide solution (0.10 M). The precipitate was extracted with 5 ml of 2-nitrotoluene and the liquid ion exchanger was dried as above.

Meperidine. The same procedure as for chlorpromazine was used, but meperidine was precipitated by mixing 2 ml of sodium tetrakis(*m*-chlorophenyl)borate solution (5×10^{-2} M) and 2 ml of meperidine hydrochloride solution (0.10 M).

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Procedures

Construction of the electrodes

An Orion liquid membrane electrode body (Model 92) was used as the electrode assembly with a Millipore LCWPO 1300 PTFE membrane; the PTFE membranes were cut to the appropriate size and a stack of four was used to avoid any leakage of the liquid ion exchanger. All the internal aqueous reference solutions were 0.010 M with respect to the cation measured and 0.10 M in NaCl. They were saturated with AgCl. The operating life of the electrodes used was about 2 months.

Preparation of calibration graphs

A 20.00-ml volume of water was pipetted into a 50-ml beaker, the electrodes were immersed in it and, after the potential had stabilised, various increments of a 0.10 M solution of the cation of interest were added. The e.m.f. readings were recorded after stabilisation following each addition, and the *E versus* log[cation] plot was constructed. The slope of the electrodes was found by regression analysis of the linear part of the graph.

Direct potentiometric assay of pharmaceutical preparations (standard additions method)

Drugs for injection. The commercial product was diluted with water so as to obtain a solution with a final concentration with respect to the active drug in the range 3×10^{-3}–3×10^{-4} M. A 20.00-ml volume of this solution was used for analysis. A potential reading was first recorded for this solution. Subsequently, a second potential reading was obtained after the addition of a small volume of a concentrated standard drug solution. The initial concentration of the sample was calculated from the change in potential.[9]

Tablets. At least five tablets were powdered and dissolved by stirring for 15 min in water. The final volume was adjusted so as to obtain a final solution concentration for the active compound in the range 3×10^{-3}–3×10^{-4} M. The resulting solution was analysed as described above by the standard additions method.

An alternative procedure was used for propantheline bromide tablets, as follows (see Results and Discussion). The powdered tablets were mixed with 25 ml of dichloromethane and stirred for 30 min at room temperature. The mixture was filtered through a sintered-glass funnel and the solid residue was washed with 10 ml of dichloromethane. The combined filtrate was evaporated to dryness by gentle heating under a stream of air. The residue was dissolved in 20 ml of water and the resulting solution was analysed as described above by the standard additions method.

Potentiometric titrations

Drugs for injection and tablets were treated as described under *Drugs for injection* and *Tablets* in order to prepare sample solutions. An 18.00-ml aliquot of the sample and 2.00 ml of buffer solution (1.00 M) were pipetted into the beaker, the electrodes were immersed in it and the titration was performed with constant stirring with either 0.01000 M tetraphenylborate or picrate solution, at a flow-rate of 0.36 ml min^{-1}. The buffers tested (0.10 M) were acetate (pH 3.3 and 5) and phosphate (pH 6). Titration curves were recorded.

Results and Discussion

Choice of Optimum Liquid Ion Exchanger

The water-insoluble salts of the cations of interest with various bulky anionic compounds dissolved in organic solvents were used as possible liquid ion exchangers for electrode construction. The slopes of the calibration graphs and the linear response ranges of various chlorpromazine electrodes are given in Table 1. It can be seen that in general the lower limit of the linear response range is smaller for electrodes containing eosin in the liquid ion exchanger. Therefore, we selected the mixture of chlorpromazine eosin with chlorpromazine tetraphenylborate dissolved in *p*-nitrocumene for further studies with the chlorpromazine electrode.

A chlorpromazine electrode has recently been reported[10] in which the membrane consists of chlorpromazine tetraphenylborate or chlorpromazine dinonylnaphthalenesulphonate in a poly(vinyl chloride) (PVC) matrix. The characteristics of these electrodes were similar to those described here. We have also constructed PVC electrodes for chlorpromazine by use of the liquid ion exchanger that contains eosin and tetraphenylborate but we did not use them routinely because of their short operating life (*ca.* 1 week) compared with 2 months or more when the liquid ion exchanger was used.

For the amitriptyline electrode we tested the amitriptyline salts with eosin, alizarin red S, tetraphenylborate, anilinonaphthalenesulphonate, eosin - tetraphenylborate mixture, eosin - tetrakis(imidazolyl)borate (all dissolved in 2-nitrotoluene), and eosin - tetraphenylborate mixture dissolved in *p*-nitrocumene, as possible liquid ion exchangers. All liquid ion exchangers were 10^{-2} M with respect to each of the dissolved salts. On the basis of the same criteria as for the chlorpromazine electrode, we selected the mixture of amitriptyline eosin with amitriptyline tetraphenylborate in *p*-nitrocumene for further study. The slope of the best amitriptyline electrode was 60 mV per decade at 25 °C and the linear working range was 6×10^{-3}–6×10^{-5} M.

No optimisation was necessary for the construction of the

Table 1. Slopes and linear response ranges for various chlorpromazine electrodes

Liquid ion exchanger*	Solvent	Slope/mV	Linear response range/M
Chl tetraphenylborate	2-Nitrotoluene	58	3×10^{-3}–8×10^{-5}
	p-Nitrocumene	60	3×10^{-3}–8×10^{-5}
Chl picrolonate	2-Nitrotoluene	46	
Chl tetrakis(*m*-chlorophenyl)borate	*p*-Nitrocumene	51	3×10^{-3}–2×10^{-4}
Chl tetrakis(imidazolyl)borate	*p*-Nitrocumene	51	4×10^{-3}–3×10^{-4}
Chl bromcresol purple	*p*-Nitrocumene	54	6×10^{-3}–6×10^{-5}
Chl eosin	*p*-Nitrocumene	62	6×10^{-3}–4×10^{-5}
	2-Nitro-*m*-xylene	53	6×10^{-3}–8×10^{-4}
	4-Nitro-*m*-xylene	51	6×10^{-3}–1×10^{-4}
Chl eosin + Chl picrate	*p*-Nitrocumene	58	4×10^{-3}–4×10^{-5}
	Decanol - *p*-nitrocumene (2 + 3)	59	5×10^{-3}–1×10^{-4}
Chl eosin + Chl tetraphenylborate	*p*-Nitrocumene	60	6×10^{-3}–4×10^{-5}

* Chl = chlorpromazine. All liquid ion exchangers were 10^{-2} M with respect to each salt dissolved.

propantheline and meperidine electrodes. Propantheline tetraphenylborate dissolved in 2-nitrotoluene (10^{-2} M) provided a suitable liquid ion exchanger. The electrode constructed had a slope of 58 mV per decade and a linear working range of 6×10^{-3}–3×10^{-6} M. Therefore, this electrode was used for further studies.

For the meperidine electrode we selected the meperidine salt of tetrakis(*m*-chlorophenyl)borate (10^{-2} M) dissolved in *p*-nitrocumene for further studies. The meperidine electrode had a slope of 60 mV per decade and a linear working range of 10^{-2}–7×10^{-6} M.

The upper concentration range of all electrodes was established experimentally and it is the point where curvature of the calibration graph begins. This curvature is, presumably, due to micelle formation.

Typical calibration graphs for the electrodes described are shown in Fig. 1.

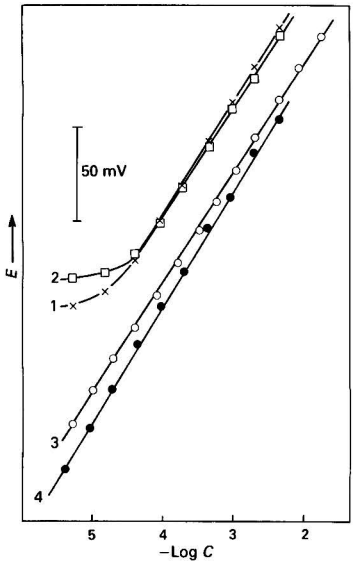

Fig. 1. Calibration graphs for (1) chlorpromazine, (2) amitriptyline, (3) meperidine and (4) propantheline electrodes

Effect of pH

The response of the four electrodes is practically unaffected by changes in pH over the pH ranges 1–6 (chlorpromazine and amitriptyline), 1–9 (propantheline) and 1–7 (meperidine). At lower acidities (higher pH) the potential changes markedly with pH because of the progressive loss of the positive charge of the molecules with increasing pH (except for propantheline, which is a quaternary ammonium compound and not a base). The potential *versus* pH graphs can be used to calculate the dissociation constant, K_a, of the cationic acid, because pK_a is equal to the pH where the initial concentration of the protonated molecule is halved, *i.e.*, when the electrode potential decreases by $0.30S$ mV (S = electrode slope).[11] With this method we calculated the pK_a of meperidine to be 8.57 ± 0.6 (at 20 °C); a value of 8.7 (at 20 °C) has been reported.[8] This graphical technique is not applicable if the unprotonated molecule is insoluble in water and precipitates at pH values lower than the pK_a (as with chlorpromazine and amitriptyline). An alternative procedure that overcomes these limitations is described elsewhere.[12]

Selectivity

The interference of various cations was studied by the mixed solution method.[9] Potentiometric selectivity coefficients are presented in Table 2. There is little interference by many common cations. Cations of similar structure or cations that form water-insoluble eosin or tetraphenylborate salts that are extractable in the organic solvents used for the preparation of liquid ion exchangers interfere seriously.

The potentiometric selectivity coefficients for amitriptyline, promethazine and thioproperazine for the chlorpromazine electrode were 0.2, 0.1 and 0.02, respectively. The K^{pot} values for chlorpromazine and perphenazine for the amitriptyline electrode were 0.4 and 0.02, respectively. Chlordiazepoxide, which is frequently present in amitriptyline and propantheline preparations, does not interfere with the electrodes at pH ≥ 6.

Propantheline is hydrolysed in solutions at pH > 5,[13] the decomposition products being xanthenecarboxylic acid and the quaternary 2-hydroxyethyldiisopropylmethylammonium cation. It was of interest to study the response of the propantheline electrode to the cationic product of hydrolysis. We hydrolysed completely a 0.01 M propantheline solution by heating in 0.1 M NaOH solution, the pH was then adjusted to 4.5 with concentrated HCl and the propantheline concentra-

Table 2. Potentiometric selectivity coefficients for the constructed electrodes

	$K_{i,j}^{pot}$			
Interferent, j	Chlorpromazine	Amitriptyline	Propantheline	Meperidine
Na^+	2×10^{-4}	3×10^{-4}	$<10^{-5}$	$<10^{-5}$
K^+	2×10^{-4}	3×10^{-4}	$<10^{-5}$	$<10^{-5}$
Li^+	2×10^{-5}	3×10^{-4}	$<10^{-5}$	$<10^{-5}$
NH_4^+	1×10^{-3}	5×10^{-4}	$<10^{-5}$	$<10^{-5}$
Mg^{2+}	5×10^{-5}	9×10^{-5}	$<10^{-5}$	$<10^{-5}$
Ca^{2+}	6×10^{-5}	1×10^{-4}	$<10^{-5}$	$<10^{-5}$

Table 3. Assay of chlorpromazine in pharmaceutical preparations by direct potentiometry (standard additions method)

	Content*				
Compound	Nominal	Found	CV, %	Reference method†	Recovery, %‡
Zuledin injection	5	5.46	2.0 ($n = 10$)	5.24	101 ± 3.5 ($n = 7$)
Largactil tablets	100	106	1.7 ($n = 10$)	99	100 ± 4.4 ($n = 5$)
Ancholactil tablets	100	94	4.0 ($n = 5$)	90	—
Antistress tablets	100	105	1.5 ($n = 5$)	—	—

* Contents are given as mg ml^{-1} for liquid preparations, otherwise as mg per tablet.

† The reference method was according to the USP.[14]

‡ With respect to amount taken. Additions were made to the drug solution, following the extraction from tablets, or directly to the diluted injection preparation.

Table 4. Assay of amitriptyline in pharmaceutical preparations by direct potentiometry (standard additions method)

	Content/mg per tablet			
Compound	Nominal	Found	CV, % ($n = 5$)	Reference method
Stelminal	25	21.3	2.6	21.1‡
Limbitrol* . .	12.5	11.2	2.0	13.5§
Saroten Retard	75	78.1	1.2	74.5§
Minitran† . .	25	25.5	3.3	30.0¶

* This preparation also contained 5 mg of chlordiazepoxide per tablet.

† This preparation also contained 4 mg of perphenazine per tablet.

‡ The reference method was according to the BP.[15]

§ The reference method was that used by the Greek National Drug Organisation, which utilises a solvent extraction step followed by spectrophotometry.

¶ This assay was based on the measurement of the chloride content by flow injection analysis. This preparation also contained 4 mg of perphenazine per tablet.

tion was determined with the electrode. We found no measurable propantheline, which suggests that the electrode only responds to the non-hydrolysed drug.

Analytical Applications

The drugs present in pharmaceutical preparations can be determined after their dissolution in water or buffer by direct potentiometry (standard additions method) or by potentiometric titration. The results of the assay of chlorpromazine in pharmaceutical preparations by direct potentiometry are given in Table 3. Zuledin injection and Largactil tablets were also analysed by potentiometric titration with sodium picrate at pH 3.3 (acetate buffer). This titrant was found to be more satisfactory than sodium tetraphenylborate because of improved precision. The results of the titrations were 5.10 mg ml^{-1} [coefficient of variation (CV) = 3.6%, $n = 3$] for Zuledin and 115 mg per tablet (CV = 0.8%, $n = 3$) for Largactil. The results suggest that the direct potentiometric methods are to be preferred to the titration methods because the titrations are more time consuming. The results for the determination of amitriptyline in pharmaceutical preparations are given in Table 4. The preparations Stelminal and Saroten Retard were also analysed by potentiometric titration with sodium picrate at pH 3.3 (acetate buffer). The results were 23.4 mg per tablet (CV = 0.6%, $n = 3$) and 73.3 mg per tablet (CV = 2.3%, $n = 3$), respectively. Chlordiazepoxide (a constituent of Limbitrol) and perphenazine (a constituent of Minitran) are weak interferents with the amitriptyline electrode. However, these compounds did not affect the assay results obtained by the electrode procedure because chlordiazepoxide was insoluble at the pH of the assay (*ca.* pH 6) and perphenazine was present at a much lower concentration than amitriptyline.

The results for the determination of propantheline in pharmaceutical preparations are given in Table 5. It was found that propantheline was not released quantitatively from the tablets by simple extraction with water or any buffer in the pH range 1–8. Therefore, we applied the extraction procedure proposed by Chatten and Okamura,[16] which yielded quantitative recoveries.

Table 5. Assay of propantheline in pharmaceutical preparations

	Content/mg per tablet			
	Nominal	Found	CV, %	Reference method*
Pro-banthine . .	15	15.9	2.1 ($n = 5$)	15.0
Flogonevrine . .	15	15.0	5.4 ($n = 4$)	19.3
Proalusin-L . .	20	21.6	2.2 ($n = 4$)	23.6

* As a reference we used a titrimetric method for halides with $AgNO_3$. The end-point was determined potentiometrically with a silver wire as indicator electrode and a double-junction Ag - AgCl reference electrode (external solution 10% NH_4NO_3). The preparations Flogonevrine and Proalusin-L also contained 2.5 and 3 mg of chlordiazepoxide hydrochloride per tablet, respectively. The higher results for these preparations obtained with the reference method are due to coprecipitation of AgCl with AgBr.

We determined meperidine by direct potentiometry in an injection preparation (0.100 g per 2 ml) and found 0.100 g per 2 ml with CV 2.3% ($n = 4$). The reference method (see Table 5) gave 0.103 g per 2 ml.

The authors thank Dr. M. A. Koupparis for the assay of the chloride content by flow injection analysis and Dr. C. Moore for providing tetrakis(*m*-chlorophenyl)borate and tetrakis-(imidazolyl)borate.

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Voltammetric Studies on a Glassy Carbon Electrode

Part II.* Factors Influencing the Simple Electron-transfer Reactions—the $K_3[Fe(CN)_6]$ - $K_4[Fe(CN)_6]$ System

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Detailed cyclic voltammetric studies were carried out on the hexacyanoferrate(II) - hexacyanoferrate(III) redox system in H_2SO_4, Na_2SO_4, NaOH, NaCl, KCl, NaH_2PO_4, $Na_2C_2O_4$ and trisodium citrate media on a glassy carbon electrode. The effects of polishing and cleaning, chemical and electrochemical surface treatments, pH, supporting electrolyte cations and anions and time on the redox behaviour of the system were systematically evaluated. The following conclusions were drawn: (a) surface polishing or exposure to the atmosphere does not affect the reproducibility of the results; (b) anodic polarisation activates the electrode surface, as found in earlier studies, but some additional evidence indicates that this is due to oxidation of the fresh carbon surface, and this aspect is considered in detail; (c) the rate constants calculated for the oxidation of hexacyanoferrate(II) and the reduction of hexacyanoferrate(III) using Nicholson's method differ substantially; this is attributed to different surface states of the glassy carbon at the starting potentials; and (d) anions have a substantial effect on both the rate constants and the limiting currents; chloride ions have a special activating effect. These effects are discussed on the basis of adsorption and mediated electron transfer. Other factors that influence the electron-transfer kinetics such as time effects and heat-treatment temperature are also discussed.

Keywords: *Voltammetry; glassy carbon electrode; simple electron transfer*

In the first electrochemical study on the glassy carbon (GC) electrode itself, Zittel and Miller[1] reported a well defined wave for the hexacyanoferrate(II) [ferrocyanide (FRO)] - hexacyanoferrate(III) [ferricyanide (FRI)] system among other reactions. This was followed by qualitative characterisation in d.c. voltammetry over a wide range of pH[2,3] and a.c. voltammetry[4] using this redox system. A few studies attempted the evaluation of the heterogeneous rate constant, k_h^0, of this reaction using Nicholson's method[5–8] and the rotating-disc electrode (RDE) method.[9] The effect of the heat-treatment temperature (HTT) of the GC electrode on the charge-transfer kinetics has been studied in detail.[9,10] The effect of potentiostatic polarisation on the redox behaviour of the FRO - FRI system was considered in a recent paper.[11]

Apart from the FRO - FRI system, a few other simple electron-transfer reactions have been studied on GC. The neptunium(VI) - neptunium(V) system was extensively studied by Plock.[12–17] The Fe^{2+} - Fe^{3+} redox system has been studied from both the analytical[1,18–20] and the kinetic[7,8] points of view. The Ce^{3+} - Ce^{4+},[1,8] Cr^{2+} - Cr^{3+}[1] and hydroquinone - quinone[7,10,11] systems have also been studied. Parson's group evaluated the rate constants of some fast systems such as nickelocene, ferrocene and $IrCl_6^{2-}$ on a GC electrode.[7] The analytical chemistry of the NADH - NAD system was considered in detail by Blaedel and Jenkins.[21,22] GC is one of the electrodes that offers the widest polarisable potential region in both the anodic and cathodic directions[23] and hence, apart from the simple electron-transfer reactions mentioned above, many other complex electrochemical reactions have also been studied on this electrode.[24,25]

In spite of the ever increasing uses of GC electrodes, there are considerable differences of opinion on the pre-treatment of electrode surface necessary to give reproducible results. Controversy exists even regarding the frequency of polishing. Polishing is suggested before recording each current - potential curve to improve electrode activity.[26–28] On the other hand, reproducibility has been claimed even after many years of use without any pre-treatment.[29] This aspect has been pointed out elsewhere.[7] Chemical cleaning in a non-oxidising medium and cathodic cycling has been suggested, as pointed out in a review.[24] Even trace amounts of oxidising agents were found to affect the reproducibility.[30] However, many recent quantitative studies indicate a better activity of an oxidised GC surface. A variety of methods for oxidation of GC have been suggested. Air oxidation above 500 °C was suggested recently.[31] Chemical oxidising agents such as nitric acid[15] and chromic acid[8] have been recommended. Electrochemical activation procedures also vary substantially. Blaedel and Jenkins[21,22] suggested potentiostatic oxidation and reduction (pre-conditioning according to their terminology) and also potentiodynamic cycling (pre-treatment) for activation of the GC surface. Potentiostatic oxidation and reduction alone were recently recommended[11] and adopted by another group.[32] Potentiodynamic cycling is often recommended but the potential ranges chosen vary widely.[4,7,33] Galvanostatic oxidation below a current density of 1 mA cm^{-2} has also been recommended.[34,35] One general pre-treatment procedure for activating GC for all electrochemical studies would be desirable, but recent papers suggest different pre-treatment procedures for different electroactive species.[11,36]

Apart from pre-treatment procedures, a few other controversies also exist regarding the GC electrode behaviour. Great care is exercised in bringing the cleaned electrode into the electrochemical cell,[7,37] but it has also been suggested that keeping the electrode in the open atmosphere activates the electrode surface.[9] There is general agreement regarding the fact that a higher heat treatment temperature (HTT) for GC results in better electrode activity,[7,38,39] but one study gave the reverse relationship.[10] The electrode activity of GC is generally believed to be less than that of Pt and greater than that of other carbon electrodes.[7,8] However, Vydra and co-workers[4,19] claimed that heterogeneous rate constants on GC electrodes are equal to or even greater than those obtained on Pt electrodes under identical experimental conditions. Even with an extremely alkaline medium reproducible results have been obtained on GC electrodes.[2,3] On the other hand, poorer results obtained at higher pH values in a few redox reactions have been attributed to poorer reproducibility of the electrode surface in alkaline media.[19,20]

In view of the above, a voltammetric study using a GC electrode with a known simple redox reaction was considered to be desirable in order to clarify some of these controversies

* For Part I, see reference 58.

and to select the proper pre-treatment procedure for further work with systems that have not been studied previously on GC electrodes and also that are more complex in nature. The FRO - FRI system was the immediate choice as it has been used to characterise many metal electrodes, carbon electrodes,[40] semiconductors[41] and even insulators.[42] In the studies cited it was generally presumed that the anionic redox species undergoes a simple electron-transfer process:

$$Fe(CN)_6^{3-} + e \rightleftharpoons Fe(CN)_6^{4-} \quad .. \quad .. \quad (1)$$

However, many examples of non-ideal behaviour have been noticed. The influence of the supporting electrolyte, cationic concentration and nature on the k_f^0 (apparent standard rate constant) of this reaction was studied by Fumkin *et al.*[43] and Kuta and Yeager.[44] Detailed studies by Peter *et al.*[45] have shown that the activated complex involved in the electron transfer is an ion pair of FRI with at least one supporting electrolyte cation. If one cation M^+ is involved in the activated complex, the process may be represented by

$$M^+ + Fe(CN)_6^{3-} \rightleftharpoons [MFe(CN)_6]^{2-} \quad .. \quad (2)$$

$$[MFe(CN)_6]^{2-} + e \rightleftharpoons [MFe(CN)_6]^{3-} \quad .. \quad (3)$$

$$[MFe(CN)_6]^{3-} \rightleftharpoons M^+ + Fe(CN)_6^{4-} \quad .. \quad (4)$$

Supporting electrolyte anions had some effect on the k_f^0 of the reaction[46] and this was attributed to the field effect. Much larger variations were noticed in a recent study[47] and were attributed to the catalytic influence of adsorbed anions (bridge effect) of the supporting electrolyte. Adsorption of the electroactive species itself has been suggested in many recent studies.[48–51] Sohr and co-workers[49,50] suggested, for example, that anionic dimers involving FRO and FRI adsorbed on the electrode function as the electroactive species:

$$Fe(CN)_6^{3-} + M^+ + Fe(CN)_6^{4-} \rightleftharpoons [Fe(CN)_6^{3-} \cdots M^+ \cdots Fe(CN)_6^{4-}]_{ads} \quad .. \quad (5)$$

$$[Fe(CN)_6^{3-} \cdots M^+ \cdots Fe(CN)_6^{4-}] \xrightarrow{e^-} 2Fe(CN)_6^{4-} + M^+ \quad .. \quad .. \quad .. \quad (6)$$

$$[Fe(CN)_6^{3-} \cdots M^+ \cdots Fe(CN)_6^{4-}] \rightarrow 2Fe(CN)_6^{3-} + M^+ + e \quad .. \quad .. \quad .. \quad (7)$$

The increase in k_f^0 observed with a decrease in concentration of the electroactive species was also attributed to the irreversible adsorption of the redox species on the electrode.[51] Radiotracer[52,53] and *in situ* spectroscopic[54] evidence has also suggested irreversible adsorption of electroactive species. In addition to the influence of solution species discussed so far, surface pre-treatment[47,55] and the surface redox state[56,57] on Pt,[9,47,56] Au[9,57] and wax-impregnated graphite[55] have also been studied.

In spite of such a detailed understanding of the non-ideal behaviour of the FRO - FRI system, it is still the best system available for electrode characterisation[40–42] because of its ready availability in pure form, stability in many solvents over a wide range of pH and reasonably well defined electrochemical behaviour. Hence in this work this redox system was used for GC surface characterisation.

Experimental

In the surface characerisation work, the background current - potential characteristics and the current - potential characteristics of the FRO - FRI system in NaCl and NA_2SO_4 media employing different pre-treatment procedures were recorded.

In the characterisation of the properties of the solution phase, a single most suitable standard pre-treatment procedure selected from the above experiments was adopted.

An H-type cell, containing a stationary glassy carbon working electrode and a Pt counter electrode in separate compartments, was used. Glassy carbon discs of diameter 5 mm and thickness 5 mm (Tokai Carbon, Japan) fixed into a glass tube using epoxy resin were used as working electrodes. Experiments in each medium were repeated with at least two or three electrodes to establish reproducibility. The saturated calomel reference electrode (S.C.E.) was connected to the working electrode through a luggin capillary. The electrolyte was deoxygenated using electrolytic hydrogen, which could be carried out either before or after placing the working electrode. The cell temperature was maintained at 25 ± 0.5 °C. All chemicals used were of analytical-reagent grade. (Merck or BDH Chemicals.) A Wenking potentiostat (75 L), a Wenking voltage scan generator (VSG-72) and a Rikadenki *x* - *y* - *t* recorder (Japan 101 T) were employed for voltammetric measurements.

Results

Surface Pre-treatment and Surface State

Polishing and cleaning

Fresh electrode samples were polished in all experiments with gradually finer emeries up to 4/0 (John Okay, UK), wiped with cotton or tissue paper to remove any solid particles attached to the electrode surface and thoroughly washed with a stream of triply distilled water. Finally, the electrode was cleaned with trichloroethylene and inserted into the electrochemical cell. Fairly reproducible results were obtained in this procedure. Further polishing with finer grade emeries and Al_2O_3 powder resulted in a small (*ca.* 10%) reduction in the background current. However, the peak current and peak shape of the FRO - FRI redox system did not vary substantially. After polishing and cleaning, the electrode was kept in the open for a few hours and then inserted into the solution. Except in the first few cathodic cycles, which are not normally recorded, this exposure to air did not have any significant influence on the voltammogram.

De-aeration of the solution is essential, as with Hg electrodes, especially when cathodic processes are studied as the dissolved oxygen gives a wave around −0.5 V *vs.* S.C.E. However, this may be carried out before or after inserting the electrode into the solution. De-aeration may be carried out with hydrogen or nitrogen.

Chemical activation

Some experiments were carried out with both 0.1 N Na_2SO_4 and Na_2SO_4 containing FRI at a constant sweep rate of 40 mV s^{-1} to ascertain the effect of chemical treatment using dilute HNO_3, dilute H_2SO_4 and dilute and concentrated chromic acid. Background currents generally increased in the order dilute H_2SO_4 < dilute HNO_3 < dilute chromic acid < concentrated chromic acid. Better defined peaks for the FRO - FRI redox system were also observed in the same order. For the same cleaning solution the background current increases with increasing time of immersion of the electrode in the cleaning solution. Beyond a critical time limit (*e.g.*, 15 s in concentrated chromic acid) the FRO - FRI peak separation increases, showing slower charge-transfer rates. If the electrode is kept in chromic acid for a longer time (>30 s) the background current increases to a level where the redox wave due to the FRO - FRI system totally disappears. Time effects are also observed (see later).

Electrochemical activation

Cyclic voltammograms of the FRO - FRI redox system with freshly polished electrodes generally show a large peak separation (ΔE_p), suggesting low electrode activity. In a few media, the current - potential graphs are less symmetrical, suggesting adsorption effects (Fig. 1). No improvement in electrode activity was observed on sweeping the electrode in the cathodic direction from the rest potential. However, the reproducibility of peak current and the peak potential improved substantially. This effect depends on the anodic

potential reached during the potential sweep. In general, anodic polarisation beyond 1.0 V in acidic and neutral media and beyond 0.6 V in alkaline media are required in order to activate the electrode surface. Subsequent cathodic reduction of the oxidised electrode does not enhance the electrode activity substantially. However, the reproducibility of the results is improved by this treatment.

Potentiodynamic sweeps were found to give more reproducible results when compared with both potentiostatic and galvanostatic anodic pre-treatments. When the electrode was kept at 1.5 V for 15 min in 0.1 N Na_2SO_4 medium containing 2 mM FRI, the peak potential separation, ΔE_p, increased, *i.e.*, the rate constant decreased. Subsequent cathodic treatment improved the reversibility of the wave, however.

In the potentiodynamic cycling, if fast sweep rates were employed (>320 mV s^{-1}), even prolonged cycling did not improve the electron transfer rate. This is especially true in the acidic and alkaline media where the oxidised surface is reduced back to a fresh carbon surface at higher sweep rates.[58]

Time effect

Specific experiments were carried out to find the time dependence of the reproducibility of electroreduction of FRI using electrodes pre-treated in different ways. The changes in peak current, peak potential, peak width and background current with increasing time were measured. Very large time effects were noted with chemically activated electrodes. The time dependence increased with the time of dipping the electrodes in the cleaning solutions. Potentiostatically polarised electrodes also showed some time dependence, this again depending on the time of polarisation and the anodic potential selected. Polished and cleaned electrodes show reproducibility at least for 15 min after dipping into the solution. Some anomalous effects, such as larger peak separation, asymmetric waves and new waves, are noticed later.

Cathodically pre-treated electrodes give much more reproducible results. Electrodes potentiodynamically cycled between anodic and sufficient cathodic potentials (−0.5 to 1.3 V in acidic media and −1.0 to 0.8 V in neutral and alkaline media) at slow sweep rates for 15–30 min generally give very reproducible results in solutions of both FRO and FRI even after 2–3 h. The electrode may be taken out, washed and kept in an air-tight container. When the same electrode without polishing and activation is used again after a few days the cyclic voltammograms show very little change in terms of peak potential and peak current.

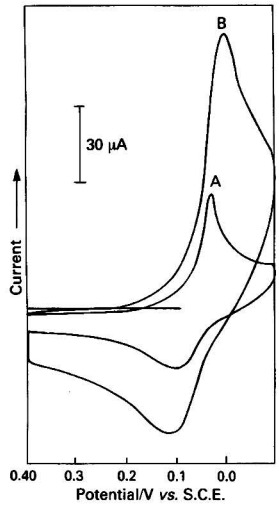

Fig. 1. Cyclic voltammogram of 2 mM FRI in 0.1 M KCl. Electrode without an electrochemical treatment. Sweep rates: (A) 10 mV s^{-1} and (B) 50 mV s^{-1}

Reduction of FRI and oxidation of FRO

The starting potentials used for recording cyclic voltammograms of FRO and FRI are different (−0.25 and 0.5 V, respectively). The surface state of GC has been shown to be different at these two potentials.[58] If any surface effects are involved in electron transfer, such effects should be reflected in the rate parameters for the oxidation and reduction of FRO and FRI, respectively. Experiments with Na_2SO_4 confirmed this expectation (Figs. 2 and 3). A substantial change in terms of peak current and peak potential was observed in NaCl medium (Figs. 4 and 5). The peak current constant values for the oxidation of FRO and reduction of FRI in these two media are substantially different (Table 1). The k_f^0 values calculated using Nicholson's method[5,6] for the oxidation of FRO and reduction of FRI are also substantially different (Table 2). In these calculations using equation (8), an α value (transfer coefficient) of 0.5 was assumed.

$$\psi = \frac{(D_{Ox}/D_R)^{\alpha/2} k_f^0}{(D_{Ox}\alpha nFV/RT)^{\frac{1}{2}}} \quad \ldots\ldots\ldots \quad (8)$$

where ψ is a function that depends on the peak separation value (ΔE_p), D is the diffusion coefficient of Ox (FRI) and R (FRO), V is the sweep rate (V s^{-1}) and all other terms have their usual meanings.

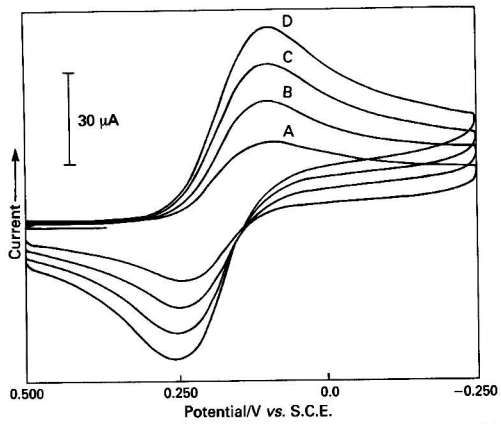

Fig. 2. Effect of concentration on the cyclic voltammogram of FRI in 0.1 N Na_2SO_4. Electrode with standard electrochemical treatment. Sweep rate: 40 mV s^{-1}. Concentration of FRI: A, 2; B, 3; C, 4; and D, 5 mM

Table 1. Peak current constants ($i_p/ACV^{\frac{1}{2}}$) for the FRO - FRI system in different supporting electrolytes (0.1 N)

No.	Medium	Oxidation or reduction	$i_p/ACV^{\frac{1}{2}}$*
1†	NaCl	Oxidation	859.8
2†	NaCl	Reduction	583.1
3†	Trisodium citrate	Reduction	526.7
4†	H_2SO_4	Reduction	470.5
5	Na_2SO_4	Oxidation	410.4
6	NaOH	Reduction	405.1
7	NaH_2PO_4	Reduction	378.9
8	Na_2SO_4	Reduction	329.3
9	$Na_2C_2O_4$	Reduction	324.1

* i_p in μA; A in cm^2; C in mM; V in V s^{-1}.

† Values correspond to lower concentrations (1–3 mM) and lower sweep rates. Values decrease with further increase in sweep rate.

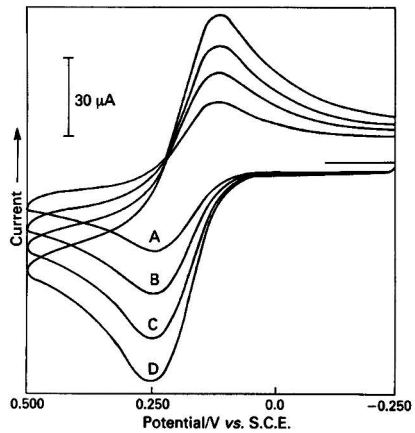

Fig. 3. Effect of concentration on the cyclic voltammogram of FRO in 0.1 N Na_2SO_4. Electrode with standard electrochemical treatment. Sweep rate: 40 mV s^{-1}. Concentration of FRI: A, 2; B, 3; C, 4; and D, 5 mM

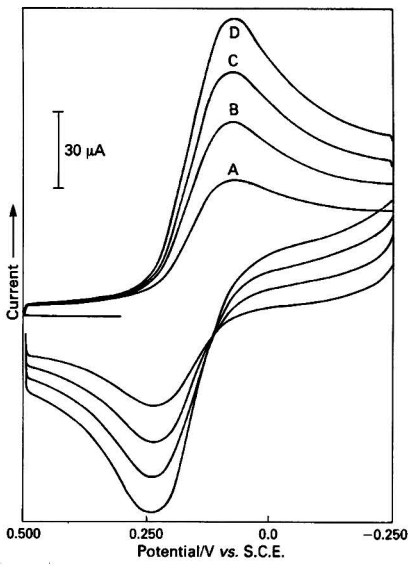

Fig. 4. Effect of concentration on the cyclic voltammogram of FRI in 0.1 N NaCl. Electrode with standard electrochemical treatment. Sweep rate: 40 mV s^{-1}. Concentration of FRI: A, 2; B, 3; C, 4; and D, 5 mM

Effect of Medium

For evaluation of the effects of the medium the electrodes were pre-treated in a standard way described above (see *Time effect*). The effect of the pH of the medium is shown in Figs. 6–8. The peak potentials are clearly dependent on the pH of the medium and the peak currents also show some variation. Cyclic voltammograms were also obtained for various sodium salts and differences in peak currents, peak potentials and peak separations were observed. Voltammograms in Na_2SO_4 and NaCl may be compared (Figs. 7 and 9). The effect of sweep rate (10–640 mV s^{-1}) and concentration of FRI (1–20 mM) were also evaluated. Typical cyclic voltammograms at different concentrations of FRI in Na_2SO_4 and NaCl media

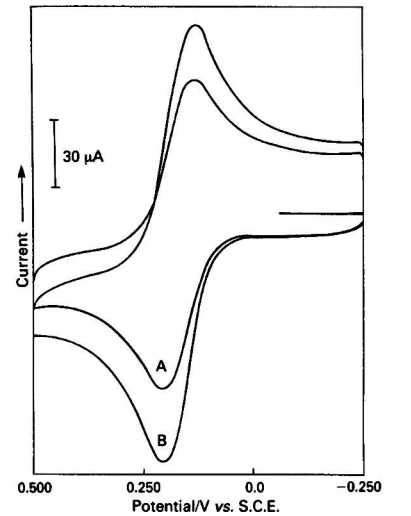

Fig. 5. Effect of concentration on the cyclic voltammogram of FRO in 0.1 N NaCl. Electrode with standard electrochemical treatment. Sweep rate: 40 mV s^{-1}. Concentration of FRO: A, 2; and B, 3 mM

Table 2. Calculation of rate constant for the FRO - FRI system in 0.1 N Na_2SO_4 medium

No.	Oxidation or reduction	Sweep rate/ $V^{\frac{1}{2}}s^{-\frac{1}{2}}$	ΔE_p/ mV	ψ	k_f^0/ $\times 10^3$ cm s^{-1}
1	Reduction	0.40	180	0.15	1.90
2	Reduction	0.2828	160	0.20	1.79
3	Reduction	0.2	140	0.26	1.65
4	Reduction	0.1414	120	0.36	1.61
5	Reduction	0.1	100	0.575	1.82
6	Oxidation	0.40	165	0.19	2.46
7	Oxidation	0.2828	140	0.26	2.38
8	Oxidation	0.2	120	0.36	2.31
9	Oxidation	0.1414	100	0.58	2.66
10	Oxidation	0.1	85	0.955	3.10

are presented in Figs. 2 and 4. The peak current for diffusion-controlled processes, either reversible or irreversible, would be proportional to $ACV^{\frac{1}{2}}$, where A is the electrode area, C is the concentration and V is the sweep rate.[59,60] Hence from peak current values the peak current constant ($i_p/ACV^{\frac{1}{2}}$) may be calculated for purely diffusion-controlled processes. Such values calculated for different media are presented in Table 1. It must be noted that when higher peak current constants are obtained (Nos. 1–4 in Table 1) they descrease slightly with increase in sweep rate.

Using ΔE_p values and the corresponding ψ functions,[5,6] electron-transfer rate constants were calculated. Typical calculations for Na_2SO_4 medium are given in Table 2. Average rate constants evaluated from such measurements in different media are presented in Table 3. As at higher concentration of electroactive species the ΔE_p values increase slightly with increasing concentration, all these rate constants were evaluated at lower concentrations of electroactive species (≤3 mM).

Discussion

GC Surface Effects on Redox Processes

Heat treatment temperature

In this work, GC material heat treated at the highest temperature (3000 °C) showed very good activity (Figs. 2–9). In earlier studies FRO - FRI and a few other systems were also

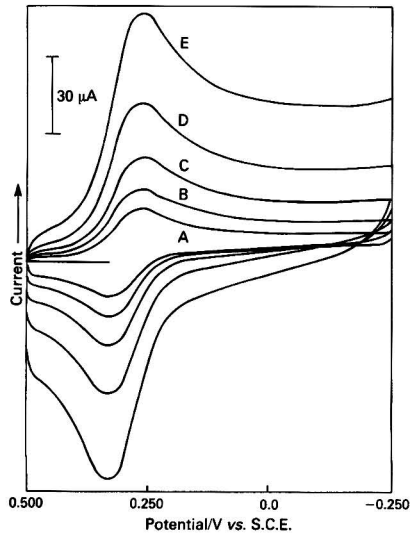

Fig. 6. Effect of sweep rate on the cyclic voltammogram of 2 mM FRI in 0.1 N H_2SO_4. Electrode with standard electrochemical treatment. Sweep rate: A, 10; B, 20; C, 40; D, 80; and E, 160 mV s^{-1}

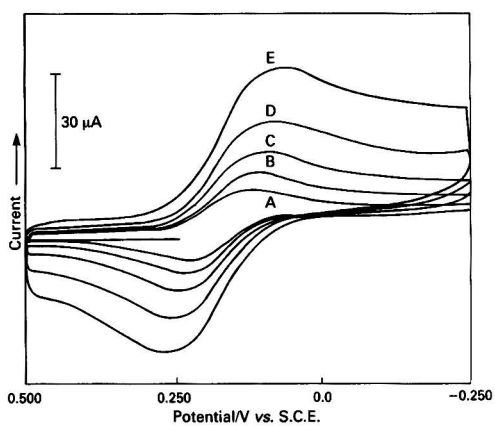

Fig. 7. Effect of sweep rate on the cyclic voltammogram of 2 mM FRI in 0.1 N Na_2SO_4. Electrode with standard electrochemical treatment. Sweep rate: A, 10; B, 20; C, 40; D, 80; and E, 160 mV s^{-1}

found to show better apparent standard rate constants as the heat treatment temperature (HTT) was increased.[7] The semiconducting properties of the electrode surface,[61] which are also related to the density of electrons in the different energy levels,[7,46] are probably responsible for this effect. This view also explains the higher rate constants noted on metal electrodes.[9,62] The decrease in apparent standard rate constant, k_R^0, in the order Pt > GC > WIG (wax impregnated graphite) > CP (carbon paste)[8] may also be explained by the decrease in the conductivity of the electrodes in this order. As pointed out earlier, it has been claimed[4] that k_R^0 on GC is equal to or greater than that on Pt. The experimental data obtained in this work (asymmetrical a.c. voltammetric wave) must be considered to be only qualitative. More quantitative evidence is needed to substantiate this aspect.

In one particular work an electrode with a higher HTT was

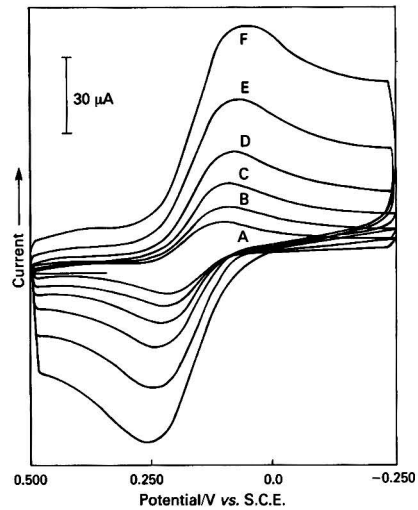

Fig. 8. Effect of sweep rate on the cyclic voltammogram of 2 mM FRI in 0.1 N NaOH. Electrode with standard electrochemical treatment. Sweep rate: A, 10; B, 20; C, 40; D, 80; E, 160; and F, 320 mV s^{-1}

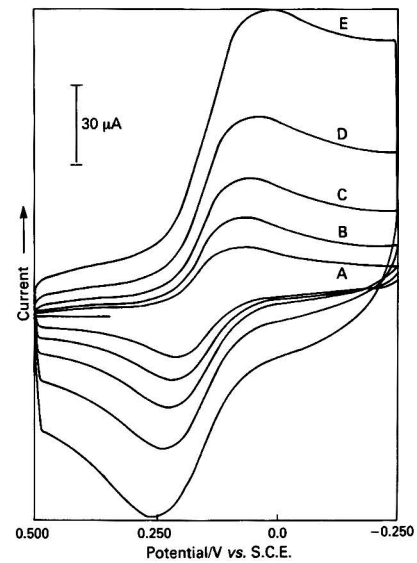

Fig. 9. Effect of sweep rate on the cyclic voltammogram of 2 mM FRI in 0.1 N NaCl. Electrode with standard electrochemical treatment. Sweep rate: A, 10; B, 20; C, 40; D, 80; and E, 160 mV s^{-1}

found to give a poor response to the FRO - FRI and also the quinone - hydroquinone system.[10] This, however, conflicts with many reports on the FRO - FRI system[2–4,7–9] and this work (Figs. 2–9). The results reported above are probably due to some surface changes such as excessive oxidation, which were probably not specifically noticed. It must also be pointed out that in a recent paper[63] the same workers reported a better response to the quinone - hydroquinone system with GC of higher HTT, in conformity with other work. Reports on the effect of the HTT of GC on other electrode processes are also available.[38,39,64]

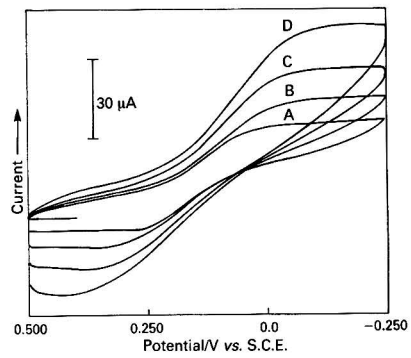

Fig. 10. Effect of sweep rate on the cyclic voltammogram of 2 mM FRI in 0.1 N trisodium citrate. Electrode after cathodic polarisation in tetraethylammonium perchlorate between 0.0 and −2.4 V. Sweep rate: A, 10; B, 20; C, 40; and D, 80 mV s^{-1}

Table 3. Average rate constants for the FRO - FRI system on a glassy carbon electrode in 0.1 N supporting electrolyte media

No.	Medium	Oxidation or reduction	k_f^0/ $\times 10^3$ cm s^{-1}	Reference
1*	H_2SO_4	Reduction	54.1	This work
2	NaCl	Oxidation	39.1	This work
3*	K_2SO_4	Oxidation	25.0	8
4*	K_2SO_4	Oxidation	16.0	7
5	K_2SO_4	Oxidation	13.2	This work
6	KCl	Reduction	6.6	This work
7	NaH_2PO_4	Reduction	3.1	This work
8	Na_2SO_4	Oxidation	2.6	This work
9	NaOH	Reduction	2.2	This work
10	Na_2SO_4	Reduction	1.8	This work
11	NaCl	Reduction	1.3	This work
12	KH_2PO_4	Reduction/ oxidation	1.3	9
13	Trisodium citrate	Reduction	1.2	This work
14	$Na_2C_2O_2$	Reduction	0.6	This work

* Supporting electrolyte concentration = 0.5 N.

Activation of GC electrodes

Electrode pre-treatments have two objectives: reproducibility and better electrode kinetics. The latter, more often called "activation of the electrode," is considered in this section and reproducibility and time effects are considered in the next section.

Even with Pt electrodes, activation of the electrode surface is still an unsettled question in both kinetic[47] and double-layer studies.[65] Activation in kinetics is believed to be achieved[66] by (a) removal of impurities and (b) metal dissolution and deposition and/or surface oxidation and reduction. With a well defined pre-treatment procedure with a GC electrode, reproducible results were obtained for longer times, *e.g.*, 2 h. Hence one must conclude that at least under the present experimental conditions the solution and the surface contained few impurities or that the impurities had little effect on the kinetics. However, in more complex reactions involving adsorbed intermediates and even in simple redox reactions where irreversible adsorption occurs at higher concentrations,[51–54] adsorption of impurities may greatly influence the electron transfer. This type of behaviour is probably responsible for the improved activity observed for ascorbic acid oxidation when the electrode was polished each time,[27,28] although the suggested catalytic effect of alumina attached on the electrode surface during the polishing treatment[27,28] cannot be easily discarded.

The question of dissolution and deposition of metal ions on metal electrodes does not arise on GC. Hence it must be concluded that the activation on GC must be due primarily to surface oxidation. This suggestion is supported by the following experimental observations. (a) Both chemical oxidation[8,12–17] and anodic polarisation bring out the same activation effect. (b) For solutions of different pH the anodic potentials required for activation are those at which fresh oxidation of surface carbon takes place.[58] (c) When the potential cycling is carried out at higher sweep rates, loosely formed surface oxides and hydroxides are reduced back to the carbon surface[58] and under this condition of pre-treatment no activation of the electrode surface is noted even on prolonged cycling. (In fact, this non-activation of electron transfer supports the conclusion in reference 58 that the surface oxides formed undergo some phase changes to form stable oxides only at slow sweep rates.) (d) Dry graphite, which contains fewer surface-active groups, has been shown to be less active than oxidised graphite electrodes.[55]

Very few attempts have been made to evaluate the thickness of the oxide layer formed on GC.[11] As pointed out earlier,[58] the answer to this question depends very much on the ability to evaluate the real (as opposed to the geometric) surface area of the electrode. However, there is compelling evidence to suggest that the oxide layer is thicker than a monolayer at least with potentiostatic oxidation for a longer time[11,67,68] and with chemical oxidations.[8] Very thick oxidised layers may, in fact, inhibit the electron transfer through the oxide layer,[57] which may explain the deactivation of the electrode surface on stronger oxidation observed in the present work. The poor reversibility reported[11] for freshly oxidised electrodes may also be due to the fact that the oxide layer formed in the oxidation was thicker than that needed for activation.

Reproducibility and time effects

As noted in the previous section, solution impurities and surface impurities contribute less to GC electrode behaviour. Surface processes are responsible for the time effects observed here.

The rest potential or open circuit potential (OCP) of cathodically treated electrodes is fairly stable (within ±10 mV). Electrodes potentiodynamically cycled under ideal conditions also show a similar OCP response. Depending on pH, the OCP measured under such conditions varies from +0.1 to −0.1 V *vs.* S.C.E. Chloride-containing media, however, give substantially different OCP values. Panzer and Elving[2] also obtained very different OCP values in KCl media. Irreversible chemisorption of chloride ions on GC electrodes may be responsible for such variations in KCl media.[69]

Electrodes potentiostatically or chemically oxidised show a substantial time dependence of the OCP, which is not reproducible. The time dependence of k_f^0 reported[8] for simple electron-transfer reactions is also closely related to this surface-state variation. In this work the rate parameters measured using chemically oxidised electrodes also showed a substantial time dependence. In Plock's work,[12–17] time dependence was avoided by dipping the electrode in dilute HNO_3 before each experiment.

Such highly oxidised electrodes may be reduced at substantial cathodic potentials (*e.g.*, −1.0 V in an acidic medium) for a longer time. The reproducibility and the activity of the electrode are improved by this treatment, as noted in this and other work.[11] The OCP behaviour also simultaneously improves. Attempts to examine the surface closely after such pre-treatments may be rewarding. In conclusion, mild oxidation or potentiodynamic sweeping is preferred to strong oxidation, at least for obtaining reproducible results. It may be noted that Taylor and Humffray,[8] who advocated chromic acid treatment, preferred mild oxidation or potentiodynamic cycling in their later work.[70]

The reduction potential used to reduce an oxidised GC is generally not very critical. Highly cathodic potentials may also

be used. However, care must be exercised when tetraalkylammonium (TAA) salts are used. If extreme cathodic potentials are used in this medium the electrode becomes deactivated (Fig. 10). The activation may not be achieved by any anodic treatment or oxidation. The electrode must be polished substantially to reactivate the surface. It seems that the reduced products of the TAA cations diffuse into the GC electrode bulk in such a situation and a hydrophobic and less conducting film is formed, causing deactivation. Diffusion of tetraalkylammonium salts and their reduction products into the comparatively more porous graphite electrode has been reported,[71] although in an entirely different context.

Influence of the oxidised and the reduced surface state on k_h^0

As stated earlier, each electrode potential applied on a GC electrode corresponds to a particular surface state. The rest potential of activated GC is normally within +0.1 V to −0.1 V (*vs.* S.C.E.), depending on the pH. Any cathodic potential from the rest potential corresponds to a reduced surface and *vice versa*. On a GC electrode, at least with respect to the FRO - FRI system, the reduced surface is found to be more active. This is probably the reason for the enhanced rate constant for the oxidation of FRO compared with the reduction of FRI in sulphate media (Table 2). A dramatic 30-fold increase in the rate constant is observed for the oxidation in comparison with the reduction in NaCl medium (Table 3, Nos. 2 and 11).

Different rate parameters for the FRO - FRI system on oxidised and reduced surfaces are not uncommon. Some reports on Pt,[47,56] Au[57] and carbon paste electrodes[55] were discussed in the Introduction. Different transfer coefficients and exchange current densities in the anodic and cathodic directions were obtained on graphite electrodes.[49,50] As pointed out in the Introduction, these facts were explained by assuming the involvement of an adsorbed dimer in the electron transfer [equations (5)–(7)]. Recently, partial charge transfer was invoked to explain this phenomenon.[72] However, it is well known that a graphite surface contains oxidised and reduced surface groups,[73–75] so it is equally probable that the variation in the surface activity of the oxidised and reduced graphite surfaces is responsible for the interesting observations observed in these studies.[49,50,75]

At least one study of GC electrodes[9] considered the effect of the direction of polarisation. RDE experiments were used to obtain the same rate constant and different transfer coefficients (0.8 for FRO oxidation and 0.2 for FRI reduction). Higher transfer coefficients reported for the anodic polarisation are in agreement with the present work, in which higher k_h^0 values were obtained for anodic oxidation assuming (for justification in assuming $\alpha = 0.5$ s) the same α value for the redox process in both directions. If alternative explanations such as adsorbed dimers[49,50] or partial charge transfer[72] are involved, this behaviour should also be observed on other solid electrodes such as Pt and Au. At least in the only study that compared the behaviour of Pt, Au and GC electrodes with respect to the FRO - FRI redox process,[9] different Tafel slopes were obtained in the anodic and cathodic directions only on the GC surface. This lends support to the present view that the anomalous behaviour observed on carbon electrodes alone[9,49,50,72] and in this work are probably due to different surface states of the carbon materials used. However, further work is needed to substantiate this viewpoint.

Effects of the Medium on Redox Processes

Effect on peak current

At low concentrations (<20 mM FRO or FRI) in all the media employed, the peak current for cathodic and anodic processes increased linearly with increasing concentration at a constant sweep rate. However, at higher concentrations (>20 mM) deviations from linearity were observed in all instances. This is probably due to irreversible adsorption of electroactive species. Such adsorption effects have also been reported earlier.[48–54] Hence all quantitative work was confined to concentrations lower than 20 mM.

At lower concentrations and lower sweep rates, the peak current was directly proportional to concentration and the square root of the sweep rate. This indicates that under these conditions the electron-transfer process is diffusion controlled. The peak current constants presented in Table 1 were obtained under these experimental conditions.

With increase in sweep rate, the current function $i_p/V^{\frac{1}{2}}$ and hence the peak current constant $I_p = i_p/ACV^{\frac{1}{2}}$ decreased in the first four instances in Table 1. This is because of the change in the redox behaviour from reversible at slow sweep rates [equation (9)] to quasi-reversible and finally to irreversible behaviour [equation (10)] at high sweep rates.[59,60]

$$I_{p,\,rev.} = 2.69 \times 10^5 n^{2/3} D_{ox}^{1/2} \quad \ldots \quad (9)$$

$$I_{p,\,irrev.} = 2.98 \times 10^5 n(\alpha n)^{1/2} D_{ox}^{1/2} \quad \ldots \quad (10)$$

Because of the presence of the factor $(\alpha n)^{1/2}$ in equation (10), the peak current value for an irreversible process is about 20% less than that for a reversible process.[60] This at least partly explains the decrease in peak current values for the first few systems in Table 1.

The peak current constant values are fairly constant even at higher sweep rates in the medium represented by the last five instances given in Table 3. The peak current constant values in these instances may be represented by either equation (9) or (10). As the k_h^0 values are also lower in these media (Table 3), it may be assumed that equation (10) is valid in this instance. The variation in peak current constant values in these instances must be due to variation in either the D_{ox} or the αn values. If the αn values are less than 0.5, the $dE_p/d\log V$ values must be greater than 60 mV.[59] For a reversible process E_p is independent of sweep rate. In all the present experiments, $dE_p/d\log V$ values between 20 and 30 mV were observed, because of the change from reversible to quasi-reversible behaviour. The above fact, however, clearly indicates that the αn value in all these instances must be close to 0.5. Symmetrical cyclic voltammograms obtained in all the media studied (Figs. 2–9) also suggest equal values in both directions, *i.e.*, close to 0.5. Hence the variation in the peak current constant values must be due to the change in D_{ox}. This indicates that some association of the electroactive species takes place in the bulk solution. These may be ion pairs[43,44] of FRO and metals ions or FRO dimers bridged by metal ions,[49,50] or even a complex species involving FRO, metal ion and supporting electrolyte anion (the FRO dimer proposed here is a bulk species present in solution, in contrast to adsorbed intermediates proposed elsewhere.[49,50] The variation in size of the resulting electroactive species is probably responsible for the variation in D_{ox} and hence the variation in peak current constants presented in Table 1.

Effect on peak potential and k_h^0

In all the media studied, well defined cyclic voltammograms were recorded. Both E_{pc} and E_{pa} shift to more cathodic potentials with increase in pH (Figs. 6–8). This is due to the acid - base equilibrium [equation (11)] in which the acidic form is the electroactive species, as suggested by Panzer and Elving.[2]

$$Fe(CN)_6^{3-} + H^+ \rightleftharpoons [HFe(CN)_6]^{2-} \quad \ldots \quad (11)$$

In neutral medium, the peak potentials did not shift substantially with variation in the nature of the supporting electrolyte if the same cation was used.

In the different media employed, the peak separation, ΔE_p, varied from 60 to 250 mV at various sweep rates of 10–640 mV s^{-1}. This permitted the calculation of k_h^0 using equation (8).[5,6] In these calculations $\alpha = 0.5$ was used. This is justified,

as pointed out in the previous section. The D_{ox} and D_R values, however, vary by about 20%, as pointed out earlier. This variation is negligible, however in comparison with the 100-fold variation in k_R^0 indicated in Table 3. Hence in these calculations the same D_{ox} and D_R values[60] were used as a first approximation.

The following conclusions may be drawn from the results in Table 3. The k_R^0 values depend very much on the cation of the supporting electrolyte. For the same sulphate anion, k_R^0 decreases in the order $H_2SO_4 > K_2SO_4 > Na_2SO_4$. This is probably due to ion-pair formation between the cation and FRO - FRI species [equations (2)–(4) and (11)]. Earlier studies on metal electrodes also showed similar effects.[43–45]

In the oxidation of FRO in the supporting electrolyte containing sodium cations, k_R^0 varied with the change of anions in the order $NaCl > Na_2SO_4$. In the reduction of FRI, k_R^0 decreased in the following order: $NaH_2PO_4 > NaOH > Na_2SO_4 > NaCl >$ sodium citrate $> Na_2C_2O_4$ (Table 3). The reason for this order cannot be clearly established with the help of the present work alone, however. The field effect of the adsorbed anions[46] or the blocking effect of the anions may be involved. At least with chloride medium the bridge effect of adsorbed chloride is involved.[47] In chloride medium, irreversible adsorption of Cl^- ions[69] may also influence the heterogeneous electron transfer. In addition to the surface effects, the electron exchange rate in solution [equation (12)] may itself depend on the supporting electrolyte,

$$Fe^*(CN)_6^{3-} + Fe(CN)_6^{4-} \overset{k_{ex}}{\rightleftharpoons} Fe(CN)_6^{3-} + Fe^*(CN)_6^{4-} \quad (12)$$

where Fe* refers to an isotope of Fe.

As according to the Marcus relationship[76] k_{ex} is related to k_R^0, the k_R^0 values may also be influenced by the medium. More detailed voltammetric work with individual media, with simultaneous adsorption measurements alone, would throw further light on this question.

Conclusions

This work clearly indicates that even for a simple reaction such as the FRO - FRI system considered here, the rate parameters depend on various factors such as cleaning procedure, electrode surface state, activation procedures adopted, the solvent, supporting electrolyte cation and anion and their concentrations and even the concentrations of electroactive species. However, from an analytical point of view the following conclusions may be drawn regarding the use of GC for simple electron-transfer studies.

(a) Polishing and cleaning and even exposure to air do not have a great influence on the electron-transfer reactions being studied, provided that a proper activation procedure is adopted. However, the background current depends quantitatively on these parameters and hence it is advisable to record background currents for each experiment at all the sweep rates to be employed.

(b) The GC surface consists of bare carbon, oxidised functions (–C═O, –COO, quinone, etc.) and reduced functions (–COH, $-CH_2OH$, hydroquinone, etc.), and the electrode activity increases in this order. Among the activation procedures adopted, a potentiodynamic sweep, which includes the bare surface oxidation region (see text) at slow rates for 15–30 min seems to be the best.

(c) The surface state depends on the potential at which the measurement is made. In sweep methods the starting potential seems to have definite influence on the kinetics. Hence it is preferable to work within specified potential regions, at least when kinetic data are collected.

(d) The anion of the supporting electrolyte has a definite influence on the electron-transfer kinetics. This aspect must be considered when using different supporting electrolytes as complexing agents or as a means of controlling pH. Within specified potential limits GC has good activity in acidic, neutral and alkaline media.

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Polarographic Assay of Nitrazepam Formulations

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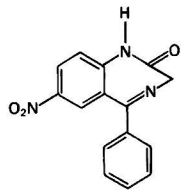
A simple, rapid, d.c. polarographic method has been developed for the determination of nitrazepam in its pharmaceutical formulations. Ethanol was employed as the solvent for extracting the drug from the formulations and five different buffer systems, namely hydrochloride (pH 1.0–2.2), McIlvaine (pH 2.2–8.0), acetate (pH 3.6–5.6), borate (pH 7.8–10.0) and Britton - Robinson (pH 2.0–12.0), were used individually as the supporting electrolyte. Nitrazepam is reduced in two-step, irreversible, diffusion-controlled and pH-dependent waves. Results obtained by the proposed method are in excellent agreement with those given in the British Pharmacopoeia. Commonly used tablet excipients were found not to interfere. A comparison was also made with different batches of nitrazepam tablets and capsules by the proposed method.

Keywords: *Nitrazepam determination; d.c. polarography; pharmaceutical formulations*

Since the introduction of chlordiazepoxide hydrochloride in 1960,[1] a large number of 1,4-benzodiazepine compounds have been investigated as tranquillisers, hypnotics, sedatives and antidepressants.[2] Nitrazepam, 1,3-dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**I**), has a more pronounced hypnotic action than other benzodiazepines and has proved to be a useful replacement for the barbiturate hypnotics.[3] Because of its relative freedom from toxic effects in the usual doses or at overdose levels and from interactions with other drugs, it may be preferred to barbiturates or non-barbiturate hypnotics.[4–6]



I

Although some reviews[2,7,8] have been published on the determination of 1,4-benzodiazepines and their metabolites in formulations and biological fluids by d.c., differential-pulse and a.c. polarographic techniques, it appears that only a single attempt[9] has been made so far to determine nitrazepam in pharmaceutical formulations. However, much work has been carried out on its determination in biological fluids.[10–13]

In recent years, high-performance liquid chromatography (HPLC)[14–16] has emerged as a more accurate technique for the determination of 1,4-benzodiazepines. However, this study represents an attempt to develop a simple, rapid d.c. polarographic method for the determination of nitrazepam in its pharmaceutical formulations. The proposed polarographic method involves a cheaper instrument than those used in HPLC and is applicable for the routine quality control of nitrazepam and other benzodiazepines in the small-scale pharmaceutical industry.

Experimental

Apparatus and Conditions for Polarographic Analysis

A manual polarograph S (Adept Laboratory, India) in conjunction with a spot galvanometer was used for the current - voltage measurements. A two-electrode combination was used, consisting of a saturated calomel electrode (S.C.E.) and a dropping-mercury electrode (D.M.E.). All the measurements were performed at 25 ± 0.2 °C. The D.M.E. had the following characteristics (in distilled water at 0.0 V open-circuit potential): $m^{2/3}t^{1/6}$ = 1.98 $mg^{2/3}s^{-1/2}$ at a mercury column height of 50 cm and an applied potential range of 0.0–1.60 V.

Controlled-potential Electrolysis

A modified H-type Lingane cell,[17] equipped with a mercury-pool cathode, S.C.E. and platinum gauze auxiliary electrode, was connected to a spot galvanometer.

A similar procedure to that described previously[18] was adopted for the controlled-potential electrolysis. The potential applied, total current passed (time) and supporting electrolyte of selected pH used are presented in Table 1.

Reagents and Solutions

All the chemicals used were either of AnalaR grade from BDH Chemicals or of general-reagent grade from E. Merck.

Five different buffer systems, namely hydrochloride (pH 1.0–2.2), McIlvaine (pH 2.2–8.0), acetate (pH 3.6–5.6), borate (pH 7.8–10.0) and Britton - Robinson (pH 2.0–12.0), were prepared in distilled water.[18] A stock solution (10^{-3} M) of nitrazepam was prepared in 40% ethanol. A 0.2% aqueous solution of Triton X-100 was used to eliminate the polarographic maxima encountered throughout the polarogram.

Reference Standard

Pure nitrazepam (99.5%) was obtained from Fairdeal Corp. (P) Ltd., India, and was used without further purification.

Table 1. Controlled-potential electrolysis parameters for nitrazepam in various buffer systems

Buffer	Selected pH	Potential applied, V *vs.* S.C.E. Wave I	Wave II	Period current passed/min Wave I	Wave II
Hydrochloride	2.00	0.60	1.20	60	40
McIlvaine	3.10	0.70	1.20	60	40
Britton - Robinson ..	3.10	0.70	1.20	60	40
Acetate	3.60	0.80	1.30	60	40
Britton - Robinson ..	11.00	1.00	1.50	40	30

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pH Dependence Studies

The polarograms of nitrazepam solutions (10^{-4} M) were recorded individually in each of the five buffer systems taken over the entire pH range and the optimum pH range, which gave well defined reduction waves in each instance, was also found.

Procedure for Studying Polarographic Behaviour

A 1.0-ml volume of the stock solution of nitrazepam was placed in a polarographic cell, 0.1 ml of Triton X-100 and 8.9 ml of appropriate buffer of selected pH were added and the solution was purged with oxygen-free nitrogen for 15 min prior to each run. The selected pH values were as follows: hydrochloride, 2.0; McIlvaine, 3.1; acetate, 3.6; borate, 8.0; and Britton - Robinson, 3.1.

Preparation of Calibration Graphs

A stock solution of nitrazepam (10^{-3} M) was prepared in 40% ethanol and solutions containing various concentrations from 1×10^{-4} to 5×10^{-4} M were prepared by dilution of the stock solution with the appropriate buffer of selected pH.

All samples were purged with oxygen-free nitrogen for 15 min prior to each scan and the stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. A similar procedure was followed for each buffer at their selected pH values. Samples with five different nitrazepam concentrations were run five times and a graph of the measured diffusion current was plotted against nitrazepam concentration.

Diffusion Dependence Studies

The polarograms of nitrazepam solution (1.0×10^{-4} M) were recorded in hydrochloride (pH 2.0), McIlvaine (pH 3.1), acetate (pH 3.6), borate (pH 8.0) and Britton - Robinson (pH 3.1) buffers at various heights of the mercury column ranging from 30 to 60 cm. The corresponding diffusion currents (i_d) obtained were plotted against the height of the mercury column (h) and values of $i_d/h^{\frac{1}{2}}$ were calculated.

Analysis of Pharmaceutical Dosage Forms

Nitrazepam was available in both tablet and capsule dosage forms. The tablet contains 5 mg of the drug and the only capsule dosage form available (Pharmaceutical Company of India, Bombay) contains 10 mg of the drug.

Assay Method

Twenty tablets were weighed and finely powdered. An amount of tablet was taken that, according to the level, would result in an approximately 10^{-3} M solution of nitrazepam. Accurately weighed nitrazepam powder was transferred into a 100-ml calibrated flask containing 40 ml of 96% ethanol. The contents of the flask were agitated for at least 30 min using a magnetic stirrer and then diluted to the mark with water. The solution was filtered through fine-pore filter-paper, discarding the first 20 ml of the filtrate. A 5-ml aliquot of the clear filtrate was pipetted into a 50-ml calibrated flask, 0.5 ml of Triton X-100 was added and the solution diluted to the mark with the appropriate buffer of selected pH. A 10-ml volume of this solution was placed in a polarographic cell and polarograms were recorded for a 0.0–1.6 V applied potential at a D.M.E. *versus* S.C.E.

The corresponding diffusion current was measured and the amount of nitrazepam per tablet was determined by comparison with calibration graphs obtained with pure nitrazepam. The experiment was repeated five times. A similar procedure was followed for each buffer system at the selected pH. For the capsule dosage form, a similar method was also adopted.

Marjan Assay Method

A standard solution (0.6 mg ml^{-1}) of nitrazepam was prepared in 40% ethanol. Twenty tablets or capsules were weighed and the average mass per dosage form was determined. A portion of the finely ground sample, equivalent to 25–35 mg of nitrazepam, was accurately weighed and transferred into a 100-ml calibrated flask containing 40 and 30 ml of ethanol and water, respectively. The contents of the flask were agitated for at least 20 min using a magnetic stirrer and then diluted to the mark with water. The solution was filtered through a fine-pore filter-paper, discarding the first 20 ml of the filtrate. A 5-ml aliquot of the clear filtrate was pipetted into a 50-ml calibrated flask, 0.5 ml of Triton X-100 was added and the solution diluted to the mark with the appropriate buffer of selected pH. A 10-ml volume of this solution was injected into a polarographic cell and polarograms were recorded after complete deaeration for 15 min for a 0.0–1.6 V applied potential at a D.M.E. *versus* S.C.E.

After obtaining the polarograms, 1.0 ml of the standard solution of nitrazepam was added to the cell, deaerated for 2 min and polarograms were recorded under the same conditions. The wave heights H and h were measured and the mass of nitrazepam per formulation was calculated using the following equation[19]:

Mass of nitrazepam per formulation (mg) =

$$\frac{ahb \times 1000}{(1.10H-h)\,W} \quad \ldots \ldots \ldots \quad (1)$$

where a (mg) is the mass of nitrazepam reference standard in 100 ml of standard solution, b (g) is the average mass of a tablet or capsule, W (mg) is the mass of sample taken for the polarographic determination, h is the wave height of nitrazepam before standard additions, H is the wave height of nitrazepam after standard additions and 1.10 is the dilution factor.

Recovery Experiments

In order to establish the reliability and suitability of the proposed method, known amounts of the pure drug were added to various pre-analysed formulations of nitrazepam and the mixtures were analysed by the proposed method.

Interference Studies

The polarograms of the drug and suitable amounts of pharmaceutical adjuvants used in the nitrazepam tablet or capsule formulations, *viz.*, starch, lactose, talc, microcrystalline cellulose and magnesium stearate, were also recorded in order to study the possible interference of excipients with the nature of the wave.

Results and Discussion

Before 1964, only limited data on the polarographic reduction of benzodiazepines had been reported, primarily concerned with the chlordiazepoxide hydrochloride. The polarography of heterocyclic compounds containing nitrogen has been discussed.[20,21] Various workers[22,23] have also studied the reduction of the azomethine group and demonstrated the reduction of the C=N moiety by controlled-potential electrolysis of 9-(*o*-iodophenyl)acridine in basic solution. The experimental information presented here indicates that nitrazepam is reduced in two-step, irreversible, diffusion-controlled and pH-dependent waves in all the buffer systems studied over the entire pH range. Well defined waves were observed in each of the buffers in a certain pH range (Table 2).

The irreversible nature of both of the waves was confirmed by logarithmic plots,[24] and for each reduction process $E_{d.e.}$ was plotted against $\log [i/(i_d - i)] - 0.546t$ and it was found that the value of slope of the above plots appreciably exceeded

Table 2. Polarographic characteristics of nitrazepam in various buffer systems. Concentration, 10^{-4} M

Buffer	Optimum pH range*	Selected pH†	$E_{\frac{1}{2}}$/V	i_d/μA	$dE_{\frac{1}{2}}/dpH$ Wave I	$dE_{\frac{1}{2}}/dpH$ Wave II
Hydrochloride	1.60–2.20	2.00	0.25	0.364	0.075	0.045
			0.86	0.624		
McIlvaine	3.00–4.00	3.10	0.32	0.374	0.064	0.059
			0.91	0.634		
Acetate	3.00–4.00	3.60	0.30	0.353	0.100	0.075
			0.93	0.613		
Borate	7.80–8.60	8.00	0.54	0.280	0.050	0.060
			1.12	0.520		
Britton - Robinson	3.00–4.80	3.10	0.31	0.374	0.075	0.043
			0.90	0.644		

* pH range giving a well defined reduction wave.
† pH at which the effects of various parameters were studied.

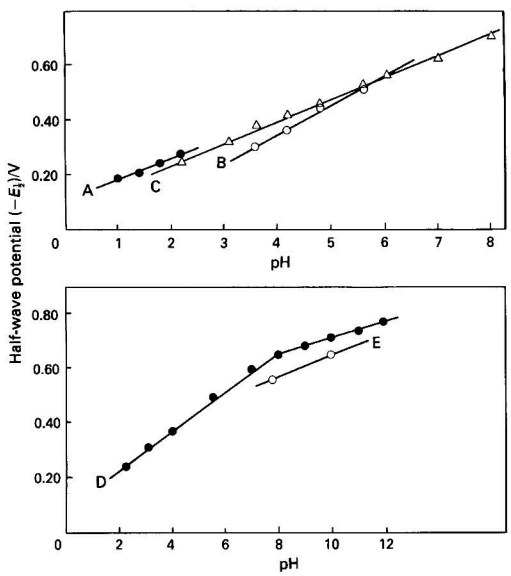

Fig. 1. Effect of pH on the half-wave potential of the first reduction wave of nitrazepam obtained in different buffer systems at a concentration of 1.0×10^{-4} M. A, Hydrochloride; B, acetate; C, McIlvaine; D, Britton - Robinson; and E, borate

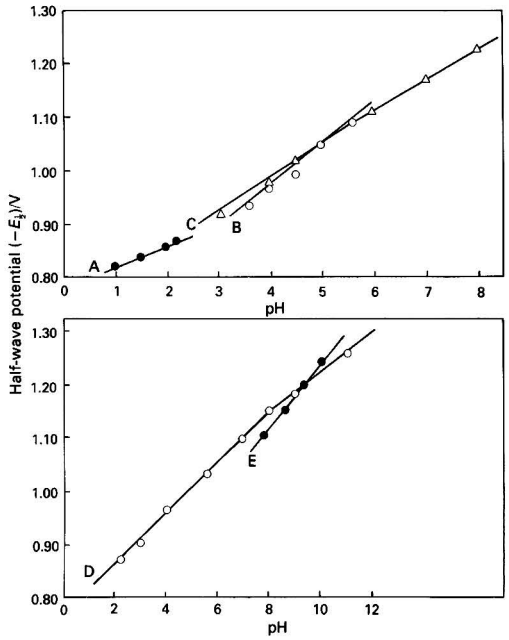

Fig. 2. Effect of pH on the half-wave potential of the second reduction wave of nitrazepam obtained in different buffer systems at a concentration of 1.0×10^{-4} M. Buffers as in Fig. 1

59.2/*n* mV. Further, the fact that $E_{1/2}$ shifted towards more negative potentials with increasing nitrazepam concentration also indicated the irreversible nature of the wave. The nature of the wave was found to be diffusion controlled, as shown by the linear dependence of the limiting current on $h_{corr}^{\frac{1}{2}}$ and depolariser concentration, constancy of the wave height over the pH range studied and the fact that d*i*/d*T* had a very low temperature coefficient.

The $E_{\frac{1}{2}}$ values of the nitrazepam reduction waves were dependent on pH and shifted towards more negative potential with increase in the pH of the buffer systems. Figs. 1 and 2 show graphs of $E_{\frac{1}{2}}$ *versus* pH for all the buffer systems studied. It is evident that a smaller shift in $E_{\frac{1}{2}}$ occurs with buffer systems with pH > 8.5.

The number of electrons involved in the over-all reduction process as determined by controlled-potential electrolysis was found to be four for both the first and the second reduction waves of nitrazepam. However, when the electrolysis experiments were performed with buffer solutions having pH > 8.5, the change in the number of electrons was four for wave I but two for wave II.

The reduction mechanism of nitrazepam in different supporting electrolytes has already been discussed.[9,25,26] Similar reduction patterns were observed in all five buffer systems studied here. Two polarographic waves were observed in the buffers with an acidic pH range. Wave I corresponded to the consumption of four electrons in the reduction of the 7-nitro substituent and wave II was due to the consumption of four electrons in the simultaneous reduction of 4,5-azomethine and hydroxylamine (formed as an intermediate in reduction of the nitro group). However, under alkaline conditions, *i.e.*, buffer systems with pH > 8.5, the identical four-electron 7-nitro reduction was followed by a mechanism corresponding only to the two-electron 4,5-azomethine reduction. It was also evident (Fig. 3, A and B) from the fact that in alkaline solution wave II is half the height of wave I, as the hydroxylamine is not reduced owing to insufficient protonation.

Both polarographic waves thus obtained in the pH ranges stated in Table 2 are well resolved and may be utilised for the

Table 3. Assay of nitrazepam dosage forms by d.c. polarography in McIlvaine buffer (pH 3.1) and by the official spectrophotometric method

Sample	Labelled amount/mg	Amount found*/mg: Calibration graph method (A)	Marjan assay method (B)	Spectrophotometric method (C)	Standard deviation/mg†: A	B	C
Tablets:							
Nitravet	5	4.8	4.9	4.9	0.03 (0.64)	0.02 (0.40)	0.05 (0.94)
Nirven	5	4.7	4.9	4.8	0.04 (0.70)	0.04 (0.85)	0.05 (0.96)
Sedamon	5	4.8	4.8	4.9	0.02 (0.58)	0.03 (0.62)	0.04 (0.89)
Hypnotex	5	4.9	5.0	5.0	0.02 (0.49)	0.03 (0.63)	0.05 (0.98)
Restorem	5	4.8	4.9	4.9	0.03 (0.70)	0.03 (0.53)	0.04 (0.85)
Capsule:							
Hypnotex	10	9.7	9.9	9.8	0.03 (0.32)	0.05 (0.50)	0.04 (0.43)

* Each value is the average of five determinations.
† Figures in parentheses are coefficients of variation (%).

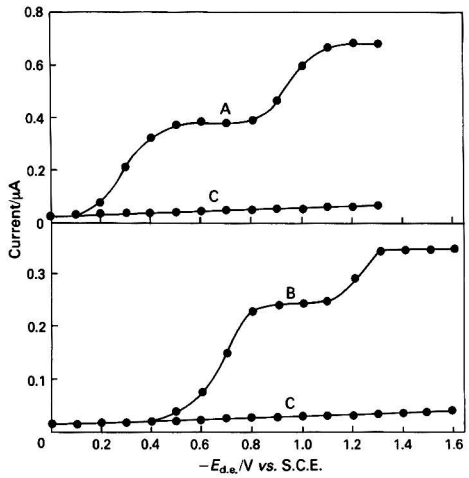

Fig. 3. Well defined reduction waves of nitrazepam obtained in different buffer systems at a concentration of 1.0×10^{-4} M. A, Acetate buffer, pH 3.6; B, Britton - Robinson buffer, pH 11.0; and C, residual current

analysis of pharmaceutical preparations, but that of wave I is highly reproducible and is therefore preferred for the analysis of tablets and capsules of nitrazepam. With the proposed polarographic method, nitrazepam can be determined at levels down to 0.27 μg ml^{-1}. Table 3 gives the assay results in McIlvaine buffer (pH 3.1) of five different batches of nitrazepam tablets and the single capsule formulation available. It also gives the results obtained using the official spectrophotometric method proposed in the British Pharmacopoeia.[27,28] The best results were observed with McIlvaine (pH 3.1), acetate (pH 3.6) and Britton - Robinson (pH 3.1) buffers for both the 5-mg tablet and 10-mg capsule preparations. In all three buffer systems analysis of the dosage forms is best performed in a moderately acidic pH range, *i.e.*, from 2.8 to 5.0. With a more acidic medium, as with hydrochloride at pH < 2.00, wave I becomes very ill-defined. However, in such circumstances, it is better to select wave II for quantitative purposes. It is also evident from Table 3 that the Marjan assay method utilising standard additions is better than the concentration - diffusion current plot method.

Table 4. Percentage recovery for nitrazepam dosage forms given by d.c. polarography in McIlvaine buffer (pH 3.1) and by the official spectrophotometric method

Sample	Labelled amount/mg	Recovery, *%: Proposed polarographic method	Official spectrophotometric method
Tablets:			
Nitravet	5	98.4	97.8
Nirven	5	98.2	98.6
Sedamon	5	97.6	98.0
Hypnotex	5	98.9	98.6
Restorem	5	97.8	98.0
Capsule:			
Hypnotex	10	98.2	98.0

* Each value is the average of five determinations.

Table 4 gives the results of recovery experiments on the nitrazepam dosage forms using the proposed method performed in McIlvaine buffer (pH 3.1) and the official spectrophotometric method. The recovery of 97.6–98.9% obtained with the proposed polarographic method indicates its accuracy and reproducibility. Also, the values are in close agreement with those of the official method. Therefore, the polarographic method as described here can safely be used as an alternative to the official spectrophotometric method. Further, the proposed method or its simple modifications shows promise for general application to a series of 1,4-benzodiazepine compounds associated with potential antidepressant, hypnotic and sedative activity.

None of the excipients commonly employed in the available nitrazepam dosage forms were found to interfere with the assay of the drug. Apparent variations in i_d/c can be produced by potential impurities (if present) that react with the electroactive substance actually responsible for the wave. These are only apparent because it is actually the concentration c that is affected in each instance, while the diffusion current may be accurately proportional to the concentration of the electroactive substance that remains.[29]

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Analytical Investigations of Cephalosporins

Part 9.* Polarographic Behaviour of Some Selected Cephalosporins and Assay of Their Formulations

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The polarographic properties of cephalothin, cephacetrile, cefamandole, cefamandole nafate and cefoperazone were investigated by using cathode-ray polarography (CRP) and differential-pulse polarography (DPP). The electroactive group present in the investigated cephalosporins is the R′ leaving group in CH_2R', which is located at the 3-position. The electrochemical methods developed were applied to cephalosporins in dosage forms and a comparison was made with the Ni(II) - hydroxylamine method. The relative standard deviations obtained for the electroanalytical methods varied between 0.28 and 0.95% and for the comparative method between 0.54 and 1.91%.

Keywords: *Polarography; cephalosporin derivatives; dosage forms; cathode-ray polarography; differential-pulse polarography*

Studies concerned with analytical methods involving the use of voltammetric determinations have gained great importance in recent years. The application of electrochemical methods to *in vitro* and *in vivo* determinations of substances bearing electroactive groups is especially advantageous, as these substances undergo no chemical change during the determination, *i.e.*, no derivatisation is involved. The polarographic methods proposed here are applicable to the initial quality control of pharmaceuticals; on the other hand the cathode-ray polarographic assay procedure has been applied to stability studies of cephalosporins and will be published in the near future.

The aim of this work was to investigate the electroanalytical properties of cephalothin, cephacetrile, cefamandole, cefamandole nafate and cefoperazone (Table 1) and to apply the electroanalytical methods developed by using cathode-ray polarography (CRP) and differential-pulse polarography (DPP) to the cephalosporins in pharmaceutical formulations.

Various methods have been proposed for the investigated cephalosporins, including titrimetric,[1-5] spectrophotometric,[6-14] fluorimetric,[15] electroanalytical,[16,17] HPLC[18-23] and microbiological methods.[24] It has been found in previous work that cephalosporins having a CH_2R' group, where R′ is the leaving group, are polarographically active.[25-27] As polarographic techniques are very precise and rapid, and by using pulse or sweep techniques the limits of determination are lowered to 10^{-7} M, it was thought appropriate to apply the developed electroanalytical method to cephalosporins in pharmaceutical formulations and to compare the results obtained with the Ni(II) - hydroxylamine method developed by Mays *et al.*[7] Optimum conditions for the proposed method for the studied cephalosporins were investigated and have been published elsewhere.[28]

Experimental

Apparatus

Polarographic measurements were carried out using a Metrohm E 506 differential-pulse polarograph and an Amel 448 A differential cathode-ray polarograph, which had a function generator and a differential vertical amplifier. For DPP operations a forced drop time of 2 s, a scan rate of 2.5 mV s^{-1} and a pulse height of 100 mV were used. The conditions of measurement for CRP were as follows: sweep amplitude, 1000 mV s^{-1}; sweep rate, 400 mV s^{-1}; and delay time, 8 s. The dropping-mercury electrode, which could be regulated electronically, had a dropping time of 26 s. Thermostatically controlled microcells (20 °C) with a saturated calomel electrode were employed. Visible spectrophotometric measurements were performed with a Beckmann B spectrophotometer using 1-cm glass cuvettes. The pH measurements were made with an Electronic Instruments 7020 instrument.

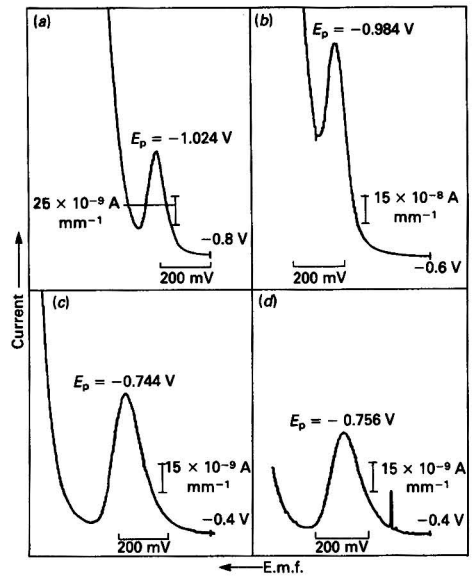


Fig. 1. Differential-pulse polarograms of (*a*) cephalothin sodium (37.3 μg ml^{-1}); (*b*) cephacetrile sodium (50.4 μg ml^{-1}); (*c*) cefamandole lithium (44.0 μg ml^{-1}); and (*d*) cefamandole nafate, measured under the conditions given in Table 2

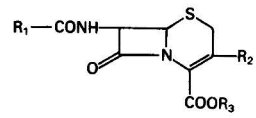
Chemicals and Reagents

Cephalosporins

Cephalothin sodium working standard and Cepovenin vials (Hoechst, FRG), cephacetrile sodium working standard and

* For Part 8 of this series, see Şengün, F. I., and Ulaş, K., *J. Pharm. Istanbul*, in the press.

Table 1. Chemical structure of the investigated cephalosporins



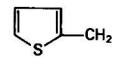
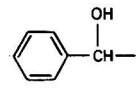
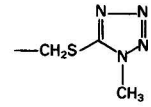
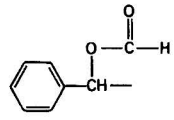
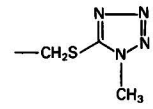
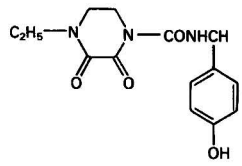
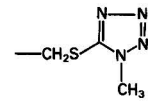
No.	Substance	R_1	R_2	R_3
1	Cephalothin sodium		$-CH_2OCOCH_3$	Na
2	Cephacetrile sodium	$N\equiv C-CH_2-$	$-CH_2OCOCH_3$	Na
3	Cefamandole lithium			Li
4	Cefamandole nafate			Na
5	Cefoperazone sodium			Na

Table 2. Experimental polarographic data for the investigated cephalosporins

Cephalosporin	Solvating agent	No. of peaks	No. of electrons	E_p	pH range for linearity	Determination limit/ ng ml^{-1} DPP	CRP
1	10% DMF - MeOH (3 + 2)	1	2	−1.024 V by citrate + HCl buffer (pH 2.0)	1.0–3.0	63	84
2	10% DMF	1	2	−0.984 V by glycine + HCl buffer (pH 1.0)	1.0–2.0	72	108
3	10% DMF	1	2	−0.744 V by citrate + HCl buffer (pH 3.0)	1.0–10.0	70	94
4	10% DMF	1	2	−0.756 V by citrate + NaOH buffer (pH 6.0)	1.0–10.0	77	103
5	10% DMF	2; 2nd peak pH > 7.0	2 + 2	1st peak, −1.088 V; 2nd peak, −1.328 V by phosphate buffer (pH 7.0)	1st peak, 1.0–10.0	67	100

Celospor vials (Ciba-Geigy, FRG), cefamandole lithium, cefamandole nafate working standards, Kefadol vials (Eli Lilly, UK), cefoperazone sodium working standard and Cefobis vials (Pfizer, FRG) and Keflin vials (Mustafa Nevzat İlaç San., Turkey) were kindly supplied for our studies.

Stock solutions used in the polarographic analyses were prepared by dissolving 10^{-3} M cephalosporin in dimethylformamide (DMF) or DMF - methanol (MeOH) (3 + 2) which was purified by column chromatography. Basic alumina (activity I) (Woelm, FRG) and silica gel 60 (0.063–0.2 mm) (Merck, FRG) were used as the column material.

Solutions of the desired concentrations were obtained by diluting the stock solutions to volume with the appropriate buffer solutions. Stock solutions should be stored below 10 °C in the dark.

Reagents and solvents

All reagents and solvents were of analytical-reagent grade. The pH values of the reaction solutions were maintained at the desired values using appropriate buffer systems. The buffer solutions used are indicated in Table 2.

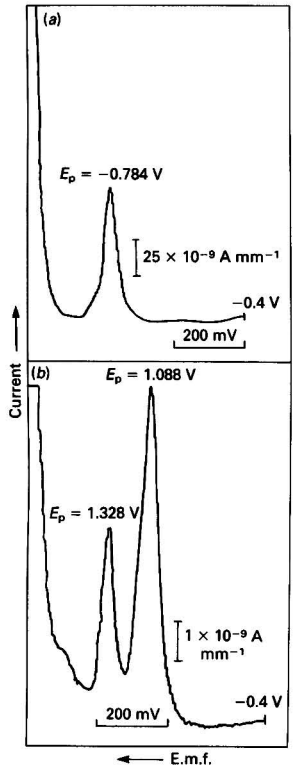


Fig. 2. Differential-pulse polarograms of cefoperazone sodium in (*a*) citrate + HCl buffer (pH 1.0) and (*b*) phosphate buffer (pH 7.0), at a concentration of 37.3 μg ml⁻¹

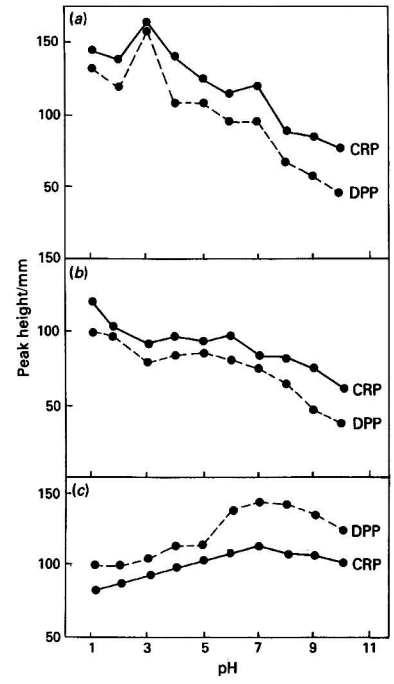


Fig. 4. Dependence of peak height on pH of (*a*) cefamandole lithium (44.0 μg ml⁻¹); (*b*) cefamandole nafate (48.8 μg ml⁻¹); and (*c*) cefoperazone (37.3 μg ml⁻¹), under the measurement conditions given in Table 2

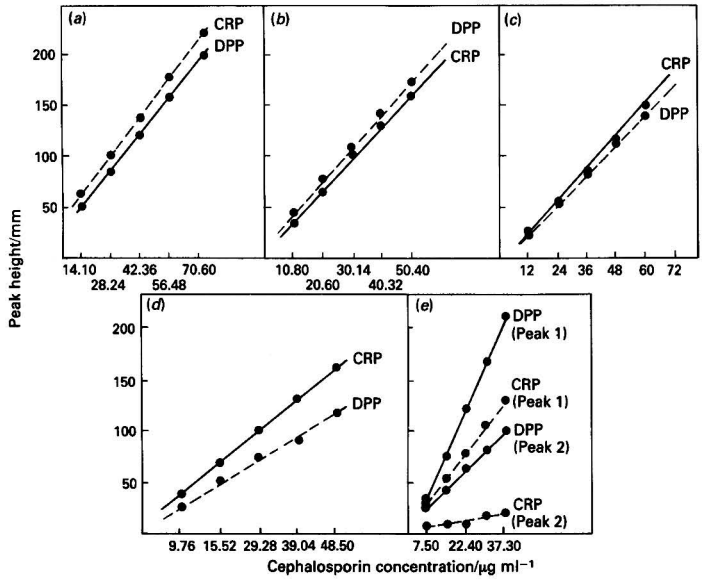


Fig. 3. Relationship between peak heights and concentrations of the cephalosporins under the measurement conditions specified in Table 2. (*a*) Cephalothin sodium; (*b*) cephacetrile sodium; (*c*) cefamandole lithium; (*d*) cefamandole nafate; and (*e*) cefoperazone sodium

Application of the Polarographic Method to Cephalosporin Vials

Pharmaceutical formulations containing 0.5, 1.0, 2.0 and 4.0 g of cephalosporin were diluted to volume with sterile water for injection solutions (according to the BP 1980), then 0.1-ml aliquots were pipetted into calibrated flasks and were diluted to 10, 25, 50 and 100 ml, respectively, with DMF or DMF - MeOH (3 + 2). From these solutions 2 ml were transferred into a water-jacketed polarographic cell kept at 20 ± 0.1 °C and 18 ml of appropriate buffer solution were added. The height of the mercury reservoir was kept at 60 cm and determinations were performed by using DPP and CRP.

Results and Discussion

In the DPP and CRP determinations of compounds 1–4 (Table 1), it was observed that they give a polarographic wave with a peak potential varying between −0.744 and −1.024 V owing to the substituted 3-methyl group, which we had previously observed in other similar cephalosporin derivatives[25–27] (Fig. 1).

The same reduction peak was observed for compound 5 in the studied pH range owing to the C-3 substituent, and another peak appeared above pH 7.0 because of the 7-[(4-ethyl-2,3-dioxo-1-piperazinylcarbonylamino) (4-hydroxyphenyl)acetylamino group (Fig. 2).

The conditions of measurement and experimental data for the studied cephalosporins that give polarographic peaks are summarised in Table 2.

The supporting electrolyte was 10% DMF or 10% DMF - MeOH (3 + 2) with the selected buffer solutions and it was found that the peak heights for all cephalosporins are linear with concentration (Fig. 3). This enables these substances to be determined in dosage forms.

No reduction peak is observed for compounds 1 and 2 above pH 3.0 and 2.0, respectively, probably owing to the interference of the reduction peak for the leaving group with the reduction potential of the cations of the supporting electrolyte. The other cephalosporins studied give a cathodic reduction peak in the pH range 1.0–10.0. The dependence of peak height (i_{sp}) and peak potential (E_p) on pH is shown in Figs. 4 and 5, respectively. The peak potentials, which are highly dependent on pH, shift to a negative value and some differences are also observed for the peak heights of the studied cephalosporins.

The reduction mechanism in Fig. 6 is proposed for the investigated cephalosporins on the basis of the results obtained after correlation with substances having similar reduction mechanisms under the same conditions, and with coulometry and controlled-potential electrolysis.

The polarographic properties determined were utilised in the assay of cephalosporins in vials, then a comparison was made with the Ni(II) - hydroxylamine method. The latter method differentiated from the methods in USP XX[29] and the Code of Federal Regulations[30] by using Ni(II) as a catalyst and stabiliser.[28] A statistical analysis for the evaluation of the methods is shown in Table 3.

As can be seen from Table 3, the relative standard deviation

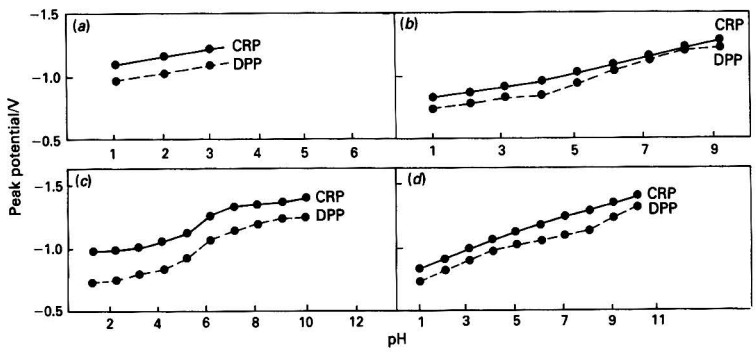


Fig. 5. Dependence of peak potential on pH of (*a*) cephalothin sodium (37.3 μg ml^{-1}); (*b*) cefamandole lithium (44.0 μg ml^{-1}); (*c*) cefamandole nafate (48.8 μg ml^{-1}); and (*d*) cefoperazone (37.3 μg ml^{-1}), under the working conditions specified in Table 2

Table 3. Statistical analysis of the results of the analysis of cephalosporin vials (n = 5)

			Amount of cephalosporin			
				Found, $\bar{x}$ ± S.D./mg		
				Polarographic		
Cephalosporin	Analysis medium	Pharmaceutical formulation (vials)	Labelled	DPP	CRP	Spectrophotometric
1	10% DMF - MeOH (3 + 2); citrate + HCl buffer (pH 2.0)	Cepovenin 1.0	1000 mg cephalothin	1003 ± 5	996 ± 8	1014 ± 6
		Cepovenin 4.0	400 mg cephalothin	4006 ± 16	3978 ± 21	4005 ± 76
		Keflin	1000 mg cephalothin	1000 ± 5	994 ± 6	996 ± 11
2	10% DMF; glycine + HCl buffer (pH 1.0)	Celospor 1.0	1000 mg cephacetrile	996 ± 4	1003 ± 9	990 ± 5
		Celospor 2.0	1000 mg cephacetrile	1990 ± 10	1969 ± 16	1991 ± 18
		Celospor 4.0	4000 mg cephacetrile	3996 ± 18	4009 ± 21	4048 ± 51
3 and 4	10% DMF; citrate + HCl buffer (pH 3.0)	Kefadol	500 mg cefamandole nafate	498 ± 1	497 ± 28	495 ± 3
5	10% DMF; acetate buffer (pH 4.6) (according to Walpole in ref. 31)	Cefobis 1.0	1000 mg cefoperazone	999 ± 4	1002 ± 7	1006 ± 9
		Cefobis 2.0	2000 mg cefoperazone	2013 ± 12	2010 ± 11	2008 ± 25

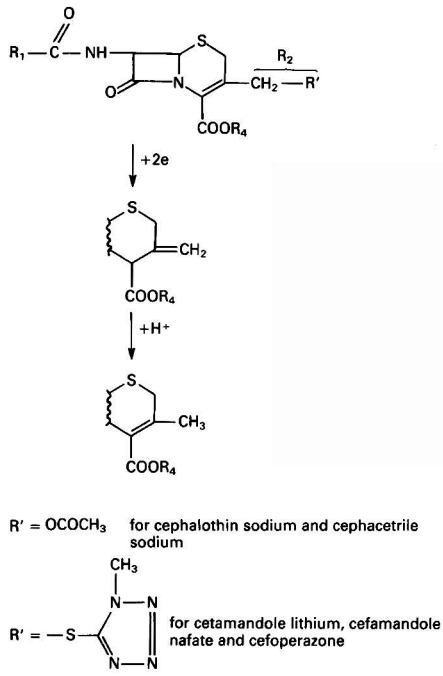


Fig. 6. Proposed reduction mechanism for the investigated cephalosporins

for DPP and CRP varied between 0.29 and 0.85%, whereas it was between 0.54 and 1.91% for the Ni(II) - hydroxylamine method.

Sincere thanks are due to the Alexander von Humboldt Foundation, FRG, for the donation of the cathode-ray polarograph.

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Determination of Chlorpromazine and its Sulphoxide in Pharmaceutical Dosage Forms by Third-order Derivative Ultraviolet Spectroscopy

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In pharmaceutical dosage forms, interference in the UV spectra of analytes by other UV-absorbing solutes leads to a requirement for the development of procedures with greater specificity. One method used to address this problem is the technique of derivative spectroscopy, where the composite spectrum is transformed to the second or higher derivative in the wavelength domain. This paper reports the novel application of this technique to the determination of a phenothiazine (chlorpromazine) and its sulphoxide impurity in various pharmaceutical dosage forms.

A systematic approach developed for optimisation of the derivative order, graphical measurement and instrumental conditions led to the adoption of third-order derivative spectroscopy as a method with suitable precision and selectivity for the determination of the phenothiazines. Both the parent compound and the sulphoxide impurity can be assayed in dosage forms by measurement of the amplitudes of, respectively, the positive peak at 259 nm with respect to the negative peak at 267 nm, and the positive peak at 350 nm with respect to the negative peak at 361 nm. A new notation for denoting these amplitude measures is proposed, *viz.*, $^3D_{259,267}$ and $^3D_{350,361}$, respectively. By comparison with an independent referee method based on reversed-phase HPLC, the proposed method and the referee method gave statistically similar results for the determination of chlorpromazine and its sulphoxide in injectables, tablets and syrups. The figures of merit for the proposed assays are described in terms of response linearity, 95% confidence limits, relative standard deviation, recovery data and correlation coefficients. It is suggested that similar methodology should be applicable to the analysis of other members of the phenothiazine class of compounds.

Keywords: *Third-order derivative UV spectroscopy; phenothiazines; chlorpromazine; chlorpromazine sulphoxide; dosage form analysis*

In recent years, the rapid evolution of microcomputers has made it possible to exploit a number of mathematical techniques that were not readily accessible before. The derivative transformation of spectral data is one example that has been shown to offer a powerful tool for both the qualitative and quantitative analysis of mixtures.[1–5] The method has found increasing application in pharmaceutical and biomedical analysis, both for background correction[2–9] and for resolution enhancement.[1,3,10] The ability to eliminate matrix interferences such as irrelevant absorption and light scattering has been of particular value.[11,12]

It is clear that the second derivative of radiation intensity becomes non-linear with concentration, except where the term ($d\varepsilon/d\lambda$) is zero.[3,4] It is common and convenient to measure absorbance, A ($\log I_o/I$), at a defined wavelength, λ:

$$A = \varepsilon bc \quad \ldots \quad (1)$$

thus

$$d^nA/d\lambda^n = d^n\varepsilon/d\lambda^n\, bc \quad \ldots \quad (2)$$

where b and c are path length and concentration, respectively, and n is the derivative order.[4]

When matrix interference can be approximated by a linear function, the first derivative yields a function where the interference is reduced to a constant:

$$y = ax + k \quad \ldots \quad (3)$$

$$dy/dx = a \quad \ldots \quad (4)$$

For total elimination of the matrix interference from the spectrum of interest, the derivative of order $n + 1$ is therefore needed, where n is the highest power of the polynomial equation which represents the matrix. Matrix UV absorption in pharmaceutical dosage forms due to adjuvants such as starch or sucrose can often be approximated by a linear function,[12,13] so that the second-derivative tranformation can be used to eliminate such interferences completely.[14]

In many formulations, however, interferences can be attributed to the presence of other UV-absorbing substances. Depending on the relative positions of the particular analytical bands concerned, polynomial equations of various degrees will be required in order to describe the interfering component effectively. Interference in many samples can often be described by a quadratic function.[12]

Such a situation is exemplified by the determination of the phenothiazine sulphoxides in the presence of their parent phenothiazine. The phenothiazines are easily converted in dosage forms by aerial oxidation into the sulphoxides, among other products.[15] The structural similarity of the sulphoxides and the parent compounds leads to difficulty in the conventional UV spectrometric methods of analysis for this group of compounds, as the UV spectra of these compounds overlap extensively, as seen for chlorpromazine hydrochloride (CPZ) and its sulphoxide (CPZSO) (Fig. 1). This invalidates the usual compendial procedure of using absorbance measurement at a single wavelength.

Several methods have therefore been proposed for addressing this problem. Some workers[16–18] have used high-performance liquid chromatography (HPLC) for various members of this class of compound. This method requires specialised equipment and expertise and can be laborious for routine analysis. Other workers[19,20] have developed difference spectrophotometric techniques, which can discriminate between the parent compound and its sulphoxide. However, these methods involve derivatisation to generate the sulphoxide itself and can involve lengthy pre-treatment of samples.

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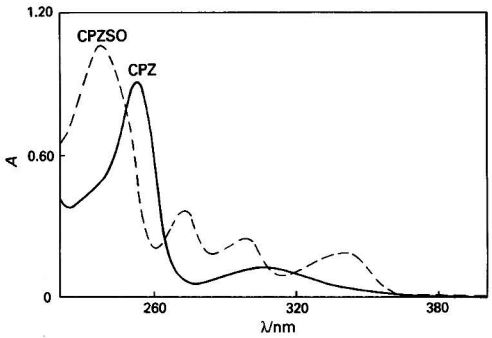


Fig. 1. Zeroth-order UV spectra of chlorpromazine (solid line) and chlorpromazine sulphoxide (broken line) in 0.1 M HCl. Instrument: HP 8450A linear photodiode array spectrophotometer

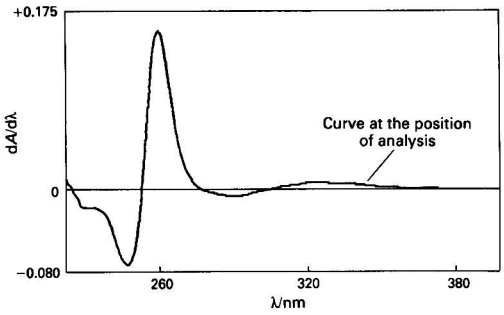


Fig. 2. First-order derivative of UV spectrum of chlorpromazine showing a broad curve at the position of the chlorpromazine sulphoxide peak at *ca.* 340–350 nm. Instrument: Perkin-Elmer (PE) Lambda-5 UV - visible spectrophotometer. The spectrum was plotted on a PE 660 thermal printer - plotter. First-derivative calculation was made on the PE 3600 Data Station

A quantitative analytical method for CPZSO based on the second derivative of its UV spectrum has been reported earlier.[21] This offered a simpler approach than, for example, the difference method for CPZSO.[20] However, a negative intercept on the *y*-axis of the calibration graph was reported, so that the technique of upper and lower bracketting standards had to be employed. In this method it was assumed that the interfering CPZ spectrum was of a quadratic nature so that the second-order derivative should substantially reduce the effect of CPZ on CPZSO. Any residual systematic error was assumed to be constant and would be eliminated by applying the graphical technique of measurement, where the amplitude of the main peak is measured with respect to that of an adjacent satellite, as discussed previously.[22] The observation of a negative intercept in the calibration procedure was, therefore, an unexpected result, which probably indicates incomplete deconvolution of the CPZ component.

Examination of the first-order derivative spectrum of CPZ near the wavelengths of graphical measurement of the CPZSO peak reveals a curve (Fig. 2), which can be approximated by a quadratic function. Hence a further two steps of differentiation should, in principle, be necessary to reduce the effect of CPZ in this region to a constant. For this reason, a derivative transformation of at least third order should be effective in deconvoluting the overlapping spectra, especially when coupled with peak-to-peak graphical measurement, when the effect of the residual constant is eliminated. This paper describes the novel application of a third-order derivative method for the determination of CPZSO in CPZ, and of CPZ itself, in pharmaceutical formulations.

Experimental

Equipment

A microcomputer-based spectrophotometer system was used, consisting of a Perkin-Elmer Lambda-5 UV - visible spectrophotometer (Bodenseewerk Perkin-Elmer GmbH, Überlingen, FRG) equipped with a thermal printer - plotter. This instrument has a scan-speed range of 15–480 nm min^{-1}. It was interfaced with a Perkin-Elmer Data Station (Model PE 3600) via a standard RS 232 C interface for storage of spectra. The Perkin-Elmer thermal printer (Model PE 660) was linked to the data station.

A second UV - visible spectrophotometer, a Hewlett-Packard (Palo Alto, CA, USA) Model HP 8450A, was configured via the RS 232 C port (9600 baud) with a Model HP 85 microcomputer, equipped with 16-kbyte additional RAM, input - output, plotter - printer, mass storage and matrix ROMs and RS 232C and HP-IB IEEE-488 interfaces. A graphics plotter (HP 7225B) and a dual 8-in flexible-disk drive (HP 9895A) were employed.

A pair of matched 10-mm silica cuvettes suitable for the far-UV region was used in this study. A Cahn Model 29 automatic electromicrobalance (Cahn USA) was used for weighing samples. An ultrasonic water-bath (Dawe Sonicleaner, Type 6443 AE) coupled with a suction system was used to aid tablet disintegration and dissolution.

The HPLC system consisted of an Altex Model 110A pump, a 100 × 5 mm i.d. column, packed with 5-μm SAS-Hypersil, and a Rheodyne injection valve fitted with a 20-μl injection loop. A variable-wavelength LC - UV detector (Pye Unicam, Cambridge, UK) was employed, equipped with an 8-μl flow cell, an absorbance scale ranging from 0.005 to 1.25 a.u.f.s. and connected to a potentiometric chart recorder (Servoscribe RE 541.20). The eluent used was CH_3CN - $(NH_4)_2CO_3$ (5 mM, pH 8.5), 75 + 25 *V/V* at 3 ml min^{-1}.

Reagents and Materials

Chlorpromazine hydrochloride (CPZ) and chlorpromazine sulphoxide (CPZSO) were obtained from May & Baker Ltd. (Dagenham, Essex, UK). Hydrochloric acid, sulphuric acid, potassium dichromate and ammonium carbonate were of analytical-reagent grade; distilled water was distilled from an all-glass apparatus; acetonitrile was of HPLC grade. The holmium oxide filter used for wavelength calibration was supplied by Perkin-Elmer. Amber-coloured or aluminium foil-wrapped calibrated flasks were used for solutions containing CPZ or CPZSO.

Methods

The spectrophotometer was ascertained to have acceptable performance by conformity with the BP standards. A solution of *ca.* 0.206 mM potassium dichromate in 0.005 M sulphuric acid was used as a test of absorbance scale accuracy. The wavelength calibration was checked using the holmium oxide filter.

Solutions

CPZ and CPZSO samples, weighed on the Cahn-29 electromicrobalance, were dissolved in 0.1 M HCl. Solutions of CPZ and CPZSO used for HPLC were prepared in 0.02 M HCl. One series of solutions was prepared containing a fixed concentration of CPZSO (1.275 μM) with various concentrations of CPZ from 10 to 50 μM; a second series was prepared containing a fixed concentration of CPZ (288 μM), with various concentrations of CPZSO from 12.75 to 90 μM. A series of calibration standards and of CPZ alone were also prepared. All solutions were prepared in amber-coloured flasks to reduce light-induced degradation of CPZ and CPZSO.

Dosage Form Pre-treatment

Syrup

In dosage form determinations, syrups of CPZ claimed to contain 5 mg ml^{-1} were diluted 1 + 4 into 0.1 M HCl by the "reverse dilution technique," where the syrup sample itself was used to make up the volume. This technique was found to give more accurate results than measurement of the viscous syrup directly with a pipette; it is useful, however, only when there is no interaction between the syrup and the diluent used. Further dilution of this sample solution was then made in the usual way. For the determination of CPZSO in dosage forms containing very low CPZSO levels, a solution of the dosage form was prepared to contain an amount equivalent to 500 μg ml^{-1} of CPZ content; 1% degradation was therefore equivalent to about 5 μg ml^{-1} of CPZSO. For the determination of CPZ, further dilution was made to give a solution of 10 μg ml^{-1} of the claimed CPZ content.

Injections

With injections, ampoules claimed to contain 25 mg ml^{-1} of CPZ were used. A 1-ml volume of the injection was diluted with 0.1 M HCl to give 500 μg ml^{-1} of CPZ for the determination of CPZSO. Further dilution was necessary for the determination of CPZ.

Tablets

Pre-treatment of the tablets to remove most of the solid insoluble tablet adjuvant was necessary. The method employed a composite of tablets: each of ten coated tablets with a label claim of 100 mg of CPZ was weighed and the average mass of each tablet determined. The tablets were then powdered in a dry glass mortar and an amount of powder equivalent to 50 mg was weighed out into a 100-ml flask wrapped with aluminium foil; 50 ml of 0.1 M HCl were added and the flask was placed in an ultrasonicator for 10 min, during which suction was applied to remove dissolved air in order to reduce any oxidation of CPZ to CPZSO. The suspended particles were then filtered with the application of suction through a Whatman sterile membrane filter of 47 mm diameter and a pore size of 0.45 μm placed on a sintered-glass support. The residue was transferred back to the flask and two further extractions with 20-ml volumes of 0.1 M HCl were carried out. The flask was rinsed and the volume made up to 100 ml. This solution was used directly for the CPZSO determination. It was diluted further for CPZ determination.

The above solutions were used for the HPLC referee method after 1 + 4 dilution with distilled water.

Selection of Optimal Instrumental Conditions for Third-order Derivative Determination of CPZSO

Measurements were made with 10-mm silica cuvettes, which were periodically cleaned in chromic acid for 72 h and thoroughly washed with distilled water. For this series of measurements, a solution of 0.0408 mM CPZSO in 0.1 M HCl was used, and graphical measurements of the peak around 350 nm were made with reference to the zero base line.

Choice of $\Delta\lambda$

At a constant scan speed of 120 nm min^{-1}, and with a slit width of 2 nm, an appropriate full-scale response was selected to permit satisfactory measurement of the noise level. Samples were scanned five times and measurements of the third-derivative amplitude were made for each value of $\Delta\lambda$, from 1 to 10 nm.

Choice of slit width

Using the same scan speed and full-scale response setting and with a $\Delta\lambda$ value of 4 nm, measurements of the third-derivative amplitude were made in replicate for each value of slit width from 0.25 to 4.00 nm. The mean noise level was also determined.

Choice of scan speed

With a slit width of 2 nm and a $\Delta\lambda$ value of 4 nm, the scan speed was varied over the range 15–480 nm min^{-1}.

Sample spectra

Spectra of all solutions were obtained on the spectrophotometer with a slit width of 2 nm at a scan speed of 120 nm min^{-1}. For the derivative spectra, the $\Delta\lambda$ value used was 4 nm. Spectra were recorded with an ordinate scale of −0.75 to 1.25 absorbance units for CPZ and −0.5 to 0.5 absorbance units for CPZSO in the third-derivative spectra.

Notation for Amplitude Measures

Graphical measurements can be expressed as previously described.[4] However, more than one derivative order may be used in a particular method, and a certain complexity is inevitably introduced in assay development when using more than one wavelength for measurement of the derivative amplitudes. The usual methods of notation[2,4] are generally imprecise.

In essence, a more generally applicable method of notation is proposed. The letter *D* is used to indicate that an amplitude of a peak has been measured in the derivative domain. The order of derivative (*e.g.*, 1, 2, 3, . . .) is specified by a leading superscript to the letter *D*, *e.g.*, ^{1}D, ^{2}D, ^{3}D. The two wavelengths between which the derivative peak is measured are specified by subscripts separated by a comma. The first wavelength corresponds to the more positive amplitude value while the second defines the position of the more negative value. Hence $^{3}D_{304,296}$ describes the peak amplitude (usually in arbitrary units) that has been measured between the more positive peak at 304 nm and the (more negative) peak at 296 nm in the third-order derivative spectrum (*cf.*, Fig. 4, peak *a*).

In those instances where a derivative peak is measured with respect to the zero base line at the same wavelength, only one wavelength need be specified, as illustrated in Fig. 4, peak *b*. Thus $^{3}D_{304}$ represents the "absolute" value of the third-order derivative at 304 nm.

Results and Discussion

In developing a derivative spectroscopic method of analysis, optimisation of all available instrumental parameters is necessary, as certain factors are known to affect the signal output. For instance, the smoothing ratio and data sampling rate have an effect on relative signal to noise ratio (SNR), depending on the full band width at half-amplitude.[23,24] Variation of the factors mentioned above will lead to signal attenuation. However, in a typical application in quantitative analysis involving the use of calibration standards, the peak-height attenuation will not in itself lead to measurement error, provided that the same method and degree of differentiation, smoothing and scan speed are used for both sample and standards. It is therefore of importance to optimise and standardise all the conditions to be used for a particular problem on a particular instrument.

In choosing the correct value of $\Delta\lambda$, the SNR at different values was considered. It is observed that the SNR increases non-linearly over the range tested (Table 1). However, the highest value of $\Delta\lambda$ (10 nm) corresponding to the highest SNR could not be used in the assay of CPZSO, because this was accompanied by considerable attenuation. A compromise had therefore to be reached. Because of the very low SNR observed, the use of $\Delta\lambda$ values of 1 or 2 nm was not considered appropriate. On increasing the value of $\Delta\lambda$ from 3 to 4 nm, the

Table 1. Variation of signal and RSD of signal with changes in $\Delta\lambda$ using 0.0408 mM chlorpromazine sulphoxide in 0.1 M HCl. The signal was measured as the amplitude of the third-order derivative peak around 350 nm to the zero base line on an arbitrary scale. Scan speed, 120 nm min^{-1}; slit width, 2 nm

$\Delta\lambda$/nm	Mean amplitude* at $^3D_{350}$	RSD	Relative amplitude†	Attenuation, %	Mean SNR*
1	0.493	10.27	100	0	0.66
2	0.172	2.07	34.9	65.1	1.30
3	0.153	2.59	31.1	68.9	4.40
4	0.142	1.18	28.8	71.2	10.91
6	0.108	0.46	22.0	78.0	42.63
8	0.079	0.56	16.2	83.8	66.70
10	0.057	0.95	11.7	88.3	113.00

* $n = 5$.

† Relative amplitudes were calculated as a percentage of that for $\Delta\lambda = 1$. Attenuation is expressed as a percentage of the amplitude at $\Delta\lambda = 1$.

Table 2. Variation of third-derivative peak amplitudes with respect to derivative zero, RSD of amplitude, SNR and $\lambda_{max.}$ of the CPZSO peak, as functions of change in slit width. $\Delta\lambda = 4$ nm; scan speed, 120 nm min^{-1}. Other conditions as in Table 1

Slit width/nm	Mean amplitude* at $^3D_{350}$	RSD	Noise†	SNR	Peak $\lambda_{max.}$/nm
0.25	0.162	10.66	0.190	0.85	349.4
1.00	0.143	1.22	0.025	5.61	349.3
2.00	0.142	1.24	0.013	10.92	349.0
4.00	0.124	0.77	0.004	30.94	349.6

* $n = 5$.

† Noise was measured as the peak-to-peak amplitude on the same scale expansion as the signal.

Table 3. Variation of third-derivative amplitude with respect to derivative zero, RSD and peak position of CPZSO as scan speed was changed. $\Delta\lambda = 4$ nm; slit width, 2 nm

Scan speed/nm min^{-1}	Mean amplitude* at *ca.* $^3D_{350}$	RSD	Actual wavelength of measurement/nm	Wavelength drift/nm
15	0.143	1.59	349.4	0.0
30	0.142	1.29	349.5	0.1
60	0.142	2.12	349.5	0.1
120	0.140	1.05	349.2	−0.2
240	0.136	0.89	348.8	−0.6
480	0.130	1.49	347.9	−1.5

* Mean amplitude was expressed on an arbitrary scale and measured for the peak around 350 nm to the zero base line ($n = 5$).

amplitude decreased by only 2.3%, corresponding to an improvement in SNR from 4.4 to 10.9. An attempt to improve the SNR further by increasing $\Delta\lambda$ to 6 nm led to a further decrease in amplitude. The value 4 nm was therefore chosen as the most reasonable value of $\Delta\lambda$ for the determination.

The choice of the most appropriate slit width was considered in a similar fashion. It can be seen in Table 2 that, as expected, there is an improvement in SNR as the slit width increases. This improvement is, however, at the expense of signal amplitude. A slit width of 2 nm was eventually chosen as giving a reasonable value of SNR with only a slight loss in signal amplitude.

Table 3 shows the changes observed in the amplitude, relative standard deviation (RSD) and peak position of the third-order derivative centroid peak as the scan speed was varied. The low RSD values indicate the high precision observed for the peaks at the scan speeds examined. Although

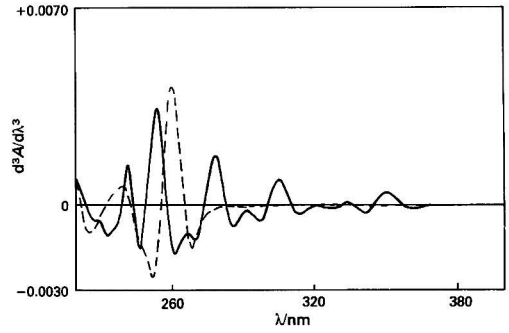


Fig. 3. Third-order derivative UV spectra of chlorpromazine (broken line) and chlorpromazine sulphoxide (solid line) overlaid to show areas of spectral overlap

Table 4. Percentage error incurred in the measurement of the third-derivative peak of chlorpromazine hydrochloride in the presence of 5% of its own mass of chlorpromazine sulphoxide

Graphical measurements	Mean amplitude* 0.03 mM CPZ	Mean amplitude* 0.03 mM CPZ plus 0.0015 mM CPZSO	Error, %†
$^3D_{259,267}$	1.266	1.266	0.0
$^3D_{259}$	1.017	1.009	0.8
$^3D_{259,252}$	1.506	1.463	2.9
$^3D_{239,252}$	0.654	0.626	4.3
$^3D_{239}$	0.165	0.172	4.2
$^3D_{239,226}$	0.392	0.406	3.6
$^3D_{218,226}$	0.590	0.600	1.7
$^3D_{218}$	0.364	0.366	0.5
$^3D_{218,208}$	0.443	0.479	8.1

* $n = 6$.

† Error (%) is determined by expressing the difference in signal amplitude relative to the single-component CPZ system.

the peak amplitude was attenuated, this was generally small and amounted to *ca.* 10% as the scan speed increased (Table 3). The position of the peak did, however, move in the direction of the scan (*i.e.*, from long to short wavelength); in this instance a shift of 1.5 nm was observed in changing the scan speed from 30 to 480 nm min^{-1}.

This shift probably reflects the fixed rate of data acquisition. The shift might therefore vary for spectra of different band widths, and could lead to a reduction in resolution of overlapping peaks. It is therefore necessary to use a scan speed that will minimise this effect. Because of the slight attenuation in amplitude, a specific scan speed should be maintained throughout the analysis of a particular sample. A scan speed of 120 nm min^{-1} was chosen for this work to give a reasonably short analysis time. At this speed, the attenuation and peak shift were low. Thus, the optimum conditions established were as follows: $\Delta\lambda$, 4 nm; slit width, 2 nm; and scan speed, 120 nm min^{-1}. These values were used throughout this work.

Assay of CPZ

Choice of assay wavelength

Fig. 1 shows the extensive overlap of the UV spectra of CPZ and CPZSO, these being taken as model compounds to exemplify the phenothiazines. The third-order derivative spectra of these compounds also overlap (Fig. 3). Characteristic peaks for the sulphoxide include those at wavelengths

Table 5. Measurements of the third-derivative signal for chlorpromazine hydrochloride using different graphical measures for binary calibration solutions simulating different degrees of degradation to chlorpromazine sulphoxide

Degradation, %, or amount present as CPZSO, % *m/m*	Concentration of CPZ in constant 0.0015 mM CPZSO/mM	Mean amplitude* $^3D_{259,267}$	$^3D_{259}$	$^3D_{218,226}$	$^3D_{218}$
13.1	0.01	0.422	0.325	0.203	0.122
7.0	0.02	0.835	0.662	0.403	0.245
4.8	0.03	1.266	0.007	0.600	0.366
3.6	0.04	1.681	1.347	0.795	0.489
2.9	0.05	2.060	1.653	0.979	0.598
0	0.03†	1.266	1.009	0.590	0.364

* $n = 5$.
† No added CPZSO.

Table 6. Least-squares regression parameters for chlorpromazine at the specified measures, in the presence of *ca.* 5% *m/m* CPZSO

Regression equation	CL*	Correlation coefficients r	r^2
$^3D_{259,267} = 41.38x + 0.013$	0.6	0.99980	0.9996
$^3D_{259} = 33.42x + 0.003$	0.8	0.99978	0.9996
$^3D_{218,226} = 19.21x + 0.0219$	0.9	0.99983	0.9997
$^3D_{218} = 11.81x + 0.011$	0.7	0.99962	0.9992

* Confidence limits in concentration ($P = 0.95$) expressed as a percentage of the central calibration concentration (0.03 mM) for chlorpromazine

around 350, 304, 277, 253, 240 and 220 nm and those for CPZ include 302, 259, 239 and 218 nm.

Derivative amplitudes at wavelengths around 259, 239 and 218 nm were considered. The peak around 203 nm was ignored because of its proximity to the stray light region and because of possible interferences from the matrix.

Table 4 shows the percentage error introduced by the presence of 5% CPZSO in the assay for CPZ (0.03 mM). The percentage error was calculated as the difference between the peak amplitude of the single-component reference solution of CPZ and that of the mixture, relative to the amplitude of the single-component solution.

Four amplitude measures for which the percentage errors for replicate measurements ($n = 6$) were less than 2% were considered further: $^3D_{259,267}$, $^3D_{259}$, $^3D_{218,226}$ and $^3D_{218}$ (Table 4). A binary calibration study and recovery (or interaction) studies were performed. The other amplitudes are appreciably affected by CPZSO derivative measures.

Binary calibration study (CPZ)

Calibration graphs were obtained for each of the graphical measures for the third-order derivatives of binary mixtures of CPZ and CPZSO. These mixtures were intended to simulate degraded pharmaceutical preparations of CPZ. It is assumed that the sulphoxidation process of CPZ yields a molar equivalent of CPZSO. Graphical measurements were made at the four pre-determined wavelengths shown in Table 5.

The binary calibration graphs were assessed according to four criteria:

(1) the linearity, as given by the correlation coefficients, r and r^2;
(2) the confidence limits in concentration ($P = 0.95$), expressed as a percentage of nominal concentration at the central calibration point for CPZ (0.03 mM);
(3) the sensitivity as given by the gradient; and
(4) the intercept.

The least-squares regression equations representing the above calibration graphs are given in Table 6, where the confidence limits (CL) and the values of r and r^2 are included for reference.

On the basis of sensitivity, it was found that the measure $^3D_{259,267}$ performed best, giving a detection limit of 0.13 μg ml^{-1} (SNR = 2), which is lower than the values reported for the difference UV spectroscopic method.[19] All the calibration graphs pass through or close to the origin.

In general, amplitudes measured with respect to the zero base line should be used with caution, as they are susceptible to drift due to any shift in the base line caused by any residual matrix interference.

In principle, such a shift can be reduced or eliminated by higher order differentiation, or simply by measuring the peak "internally" with respect to another feature in the derivative spectrum, as discussed above (Fig. 4). The RSD ($n = 6$) values for the central calibration point (CPZ, 0.03 mM; CPZSO, 0.0015 mM) were as follows: $^3D_{259,267}$, 0.21; $^3D_{259}$, 0.22; $^3D_{218,226}$, 0.0; and $^3D_{218}$, 0.22%. This confirms the high precision of the method.

Interaction study (recovery of CPZ)

The effect of CPZSO on the assay of CPZ was evaluated by examining the percentage recoveries of CPZ when in mixtures that simulate different levels of degradation. The amount of CPZ recovered was compared with the stoicheiometric amount and plotted for the different amplitudes studied, as illustrated in Fig. 5 for one amplitude. In every instance the recovery was close to quantitative.

In considering all the above-mentioned factors, the preferred measure for the assay of CPZ in the presence of CPZSO was found to be $^3D_{259,267}$.

Table 7. Percentage error incurred in the measurement of chlorpromazine sulphoxide in the presence of more than 600% of its own mass of chlorpromazine hydrochloride

Graphical measurements	Mean amplitude* 0.0432 mM CPZSO	0.0432 mM CPZSO plus 0.288 mM CPZ	Error, %†
$^3D_{304,340}$	0.402	0.409	1.7
$^3D_{304,296}$	0.493	0.556	12.8
$^3D_{304}$	0.307	0.314	2.6
$^3D_{350,361}$	0.197	0.198	0.5
$^3D_{350,340}$	0.244	0.237	2.9

* $n = 5$.
† Error (%) is expressed as the difference in signal of the single-component and that of the two-component mixture, relative to the signal of the single-component system.

Table 8. Measurements of the third-derivative signal for chlorpromazine sulphoxide using different graphical measures for a binary calibration study simulating different degrees of degradation of chlorpromazine to its sulphoxide

Degradation, % (*i.e.*, % present as CPZSO)	Concentration of CPZSO in constant 0.288 mM CPZ/mM	Mean amplitude* $^3D_{304,340}$ (*d*)	$^3D_{304}$ (*b*)	$^3D_{350,361}$ (*f*)	$^3D_{350,340}$ (*e*)
1.2	0.0036	0.039	0.030	0.018	0.018
3.6	0.0108	0.113	0.089	0.052	0.053
7.0	0.0216	0.205	0.160	0.099	0.113
13.0	0.0432	0.408	0.316	0.198	0.237
18.4	0.0648	0.612	0.475	0.289	0.357
100.0	0.0432 (no CPZ)	0.402	0.307	0.197	0.244

* $n = 5$. Letters in parentheses correspond to the peak codes in Fig. 4.

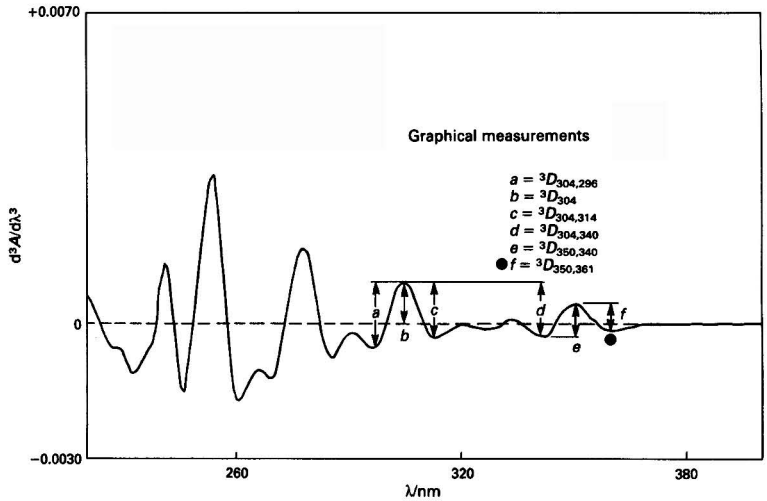


Fig. 4. Third-order derivative spectrum of chlorpromazine sulphoxide. Some of the possible graphical measures are indicated. The proposed method of unambiguous annotation for derivative amplitude measures is also listed. Measurement *f* was used in the quantitation of chlorpromazine sulphoxide

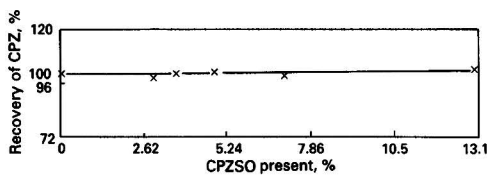


Fig. 5. Interaction curve showing the recovery of chlorpromazine HCl for a fixed concentration of chlorpromazine sulphoxide (0.0015 mM) using measurements at $^3D_{259,267}$

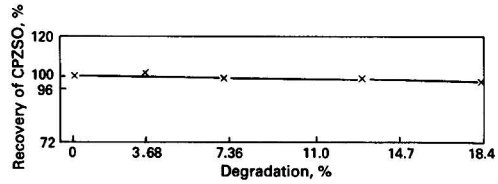


Fig. 6. Interaction curve showing the recovery of chlorpromazine sulphoxide from a fixed concentration of chlorpromazine HCl (0.288 mM) using measurements at $^3D_{350,361}$

Table 9. Least-squares regression parameters for chlorpromazine sulphoxide in the presence of CPZ for the specified measures

Regression equation	CL*	Correlation coefficients r	r^2
$^3D_{304,340} = 9.34x + 0.007$	3.8	0.99945	0.9989
$^3D_{304} = 7.13x + 0.009$	3.0	0.99958	0.9992
$^3D_{350,361} = 4.4x + 0.005$	1.4	0.99980	0.9996
$^3D_{350,340} = 5.63x + 0.008$	3.5	0.99967	0.9993

* Confidence limits in concentration (P = 0.95) expressed as a percentage of the central calibration concentration (0.0432 mM) for chlorpromazine sulphoxide with 0.288 mM of CPZ.

Assay of CPZSO

Choice of assay wavelength

Preliminary studies of the spectra of CPZ and CPZSO, illustrated in Fig. 3, revealed that the peaks available in the third-order derivative for CPZSO occurred at wavelengths around 350, 304 and 277 nm. The use of the peak near 277 nm was not considered further because of gross interference by an adjacent CPZ peak. Further preliminary analysis of the performance of the various graphical measures was carried out on the basis of an interaction study on the spectra of the following solutions: 0.0432 mM CPZSO alone in 0.1 M HCl, and a solution of 0.0432 mM CPZSO with 0.288 mM CPZ in 0.1 M HCl (simulating about 12.5% degradation of CPZ).

Amplitudes were recorded for the different measures summarised in Table 7 and the percentage error involved in the determination of CPZSO due to the presence of CPZ was determined, as described earlier for CPZ The following amplitudes were chosen for further consideration, because of the relatively low percentage error observed in the presence of CPZ: $^3D_{304,340}$, $^3D_{304}$, $^3D_{350,361}$ and $^3D_{350,340}$.

Binary calibration study (CPZSO)

A binary calibration study of CPZSO in CPZ was performed. Table 8 shows the mean amplitude for the graphical measures selected. The binary calibration graphs were assessed as described above for those of CPZ; the least-squares regression equations are shown in Table 9. Taking into account the intercept and the confidence limits, it is concluded that the graphical measure $^3D_{350,361}$ should give the most satisfactory measure for quantitative analysis.

The precision of the response was studied at the central calibration concentration of CPZSO, 0.0432 mM. The RSD values for the four amplitides selected were as follows: $^3D_{304,340}$, 1.05; $^3D_{304}$, 0.85; $^3D_{350,361}$, 0.25; and $^3D_{350,340}$, 0.21%. This confirms the high precision attained in measuring the amplitude by this method.

Interaction studies (recovery of CPZSO)

The effect of high concentrations of CPZ on the determination of CPZSO was evaluated by determining the percentage recovery of CPZSO in binary mixtures. The amount of CPZSO recovered is shown as a percentage of the stoicheiometric amount. The percentage recovery plotted as a

Table 10. Comparison of results obtained by third-order UV derivative spectroscopy and a referee method based on reversed-phase HPLC, applied to the analysis of various dosage forms of chlorpromazine and its degradation product, chlorpromazine sulphoxide

	Dosage form							
	Tablets*		Injectable†		Syrup 1‡		Syrup 2§	
	I¶	II¶	I	II	I	II	I	II
Percentage of claimed content	99.9	100.2	99.3	99.8	98.3	97.7	81.4	82.2
RSD‖	0.95	0.38	0.64	0.21	0.55	0.25	0.58	0.44
Calculated t**	0.39		1.16		1.99		1.15	
Calculated F††	1.95		5.43		4.69		4.32	
Amount of CPZSO found in 100 μg equivalent to CPZ dosage form/μg	0.39	0.40	0.83	0.86	2.90	2.85	15.74	15.04
RSD‖	23.90	6.30	11.10	3.10	1.70	3.05	0.34	0.56
Calculated t**	0.25		0.53		0.40		2.41	
Calculated F††	1.95		5.43		4.69		4.32	
CPZ degraded, %	0.41	0.43	0.88	0.91	3.08	3.02	16.7	15.96

* Coated tablets containing 100 mg of chlorpromazine hydrochloride.
† Injection ampoules containing 25 mg ml^{-1} of chlorpromazine hydrochloride.
‡ Unexpired chlorpromazine syrup (opened for the first time) (5 mg ml^{-1}).
§ Expired chlorpromazine syrup that had been opened previously containing 5 mg ml^{-1}.
¶ I, HPLC (referee) method; II, third-order derivative UV spectroscopy method.
‖ $n = 4$.
** Tabulated value of $t = 2.45$ ($P = 0.05$).
†† Tabulated value of $F = 9.28$ ($P = 0.05$).

function of the equivalent percentage degradation is shown in Fig. 6.

Hence for further applications, it is suggested that measurement at $^3D_{350,361}$ be employed for determining CPZSO in CPZ.

Application to Dosage Forms

The final conditions established above for the assay of CPZ and CPZSO in pharmaceutical dosage forms gave results in excellent agreement with those obtained using an independent referee method based on reversed-phase HPLC[18] (Table 10). The relative standard deviation shown in Table 10 exemplifies the high precision of the proposed method using the microcomputer-based spectrophotrometer; the higher RSD observed at low concentrations of CPZSO when determined with the HPLC method indicates the difficulties involved when very small signals have to be measured. Student's t-tests applied to the results of each of a series of dosage forms showed that the means for each were comparable (Table 10) and that any difference in the variance ratio was attributable to the greater variability of the HPLC referee method.

The proposed method clearly detects the extensive degradation that has occurred in syrup 2 (Table 1). The mean values ($n = 4$) for both CPZ and CPZSO agree very well with the referee method. It should be noted, however, that decomposition of phenothiazines can be fairly complex and will depend on the storage conditions. For example, decomposition products of phenothiazine when exposed to light and air, or when exposed to air alone in the dark, differ both qualitatively and quantitatively.

In this study, adequate precautions were taken to protect samples from photodegradation. Most commercial phenothiazine dosage forms are normally protected from light. However, syrups during use are frequently exposed to atmospheric oxygen, as exemplified by syrup 2. Under these circumstances, the phenothiazine sulphoxide is a preferred degradation product. This would explain the high values of CPZSO observed when using either the proposed method or the HPLC referee method. It can therefore be reasonably concluded that under these circumstances, the selectivity of the proposed method is adequate for the routine control of CPZ in pharmaceutical formulations throughout the shelf-life of the product.

Conclusions

The use of derivative spectroscopy for the quantitative analysis of closely related analytes requires the careful selection of the appropriate analytical bands, the appropriate derivative order and optimisation of all relevant instrumental parameters. It is difficult to select the appropriate derivative order for quantitative determination on the basis of visual examination of the overlapping spectra alone. Although many earlier applications of the technique have used the second-order derivative for spectral deconvolution, in some instances a higher derivative order may be necessary, as exemplified in this work.

If the matrix interference can be approximated by a quadratic function, this can be substantially reduced by employing second-order differentiation, coupled with measurement of the main peak with respect to a satellite peak. In such instances, the third-order derivative, where the amplitude is simply measured directly with respect to the base line can give signals that are proportional to concentration.

Many workers in this field have advocated the use of even-order derivatives because of the coincidental occurrence of the centroid peak with the zero-order band peak.[7] In certain instances the use of the next higher even order derivative (*i.e.*, the fourth derivative) may not be practicable, because of the very low peak amplitude observed, as is the case here for the determination of CPZSO. As noted by Talsky *et al.*,[3] the odd-order derivatives should, however, be equally useful in practice.

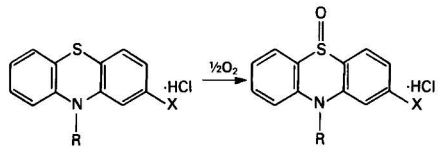
The use of the third-order derivative has been found to be the most appropriate in the present instance. The minimum detectable amount of CPZSO in the unsmoothed spectrum is *ca.* 0.64 μg ml^{-1} (SNR = 2). The method can be readily extended to the assay of low levels of CPZSO (<1%) by using large amounts of the drug sample for measurement (500 μg ml^{-1}), at which the absorbance above 300 nm can still be measured accurately.

It was found that degradation as low as 0.13% of CPZSO in dosage forms of CPZ could be detected by the proposed method. However, CPZSO must be assayed at wavelengths above 300 nm, as absorbances higher than 3 are observed at lower wavelengths. The minimum measurable amount of CPZSO using the fourth-order derivative at around 340 nm (measured with respect to the derivative base-line zero) was

found to be *ca.* 1 μg ml^{-1} (SNR = 2). This corresponds to 0.2% degradation in CPZ dosage form when using 500 μg ml^{-1} of the drug sample. The noise level was significantly higher in the fourth-derivative order measurements.

The determination of CPZSO by the proposed method exploits the band near 340 nm, which presents a relatively sharp feature under the envelope of the CPZ spectrum when CPZ is present in large amounts.

The transformation



	R	X
Chlorpromazine	$(CH_3)_2N(CH_2)_3$	Cl
Promethazine	$(CH_3)_2N(CH_3)CHCH_2$	H
Trimeprazine	$(CH_3)_2NCH_2(CH_3)CHCH_2$	H

imparts the same chromophoric effect to most members of the group, leading to an absorption peak near 340 nm due to the red shift of *ca.* 30 nm of a fundamental band of the parent chromophore. Some of the common phenothiazine derivatives that share this property include triofluoperazine, thioproperazine, levopromazine, fluphenazine and promazine. The proposed method should therefore be generally applicable to the determination of these phenothiazines.

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Spectrophotometric Determination of Certain Sympathomimetic Amines

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The chromogenic agents *p*-anisaldehyde and *p*-tolualdehyde were used for the determination of the sympathomimetic amines methyldopa and noradrenaline. A purple colour developed when an alkaline solution of methyldopa and noradrenaline was allowed to react with *p*-anisaldehyde or *p*-tolualdehyde at pH 4.7. Beer's law was obeyed over the ranges 0.17–1.35 mg of noradrenaline and 0.24–1.68 mg of methyldopa. Benzaldehyde was also used for the determination of sympathomimetric amines. The results are compared with those obtained with official methods.

Keywords: *Spectrophotometry; p-anisaldehyde; p-tolualdehyde; benzaldehyde; sympathomimetic amines*

Several procedures have been described for the determination of sympathomimetic amines, involving titrimetry and spectrophotometry,[1–3] spectrofluorimetry,[4] gas - liquid chromatography[5,6] and polarography.[7]

In this work, aromatic aldehydes were used as chromogenic reagents for the determination of noradrenaline and methyldopa. The reaction was carried out with acetate buffer at pH 4.7 and the purple colour formed was measured at 540 nm. The purpose of the investigation was two-fold: to develop a simple, rapid and more convenient method for the spectrophotometric determination of noradrenaline and methyldopa, especially for the determination of noradrenaline in the presence of adrenaline, and to apply the procedure to pharmaceutical preparations.

Experimental

Instruments

A Unicam SP 1800 spectrophotometer and a Pye Unicam Model 292 pH meter, equipped with a No. 529560 glass electrode, were used.

Reagents

All the chemicals used were obtained from E. Merck.

p-Anisaldehyde, 0.2% *m/V solution in methanol.*

p-Tolualdehyde, 0.2% *m/V solution in methanol.*

Benzaldehyde, 0.2% *m/V solution in methanol.*

Sodium hydroxide solution, 1.0% *m/V.*

Acetate buffer solution (*pH* 4.7). Prepared by mixing equal volumes of 0.01 M acetic acid and 0.01 M sodium acetate solution and adjusting the pH using a pH meter.

Sympathomimetic amine solutions. Separate solutions of L-adrenaline, L-noradrenaline and methyldopa (BDH Chemicals) of concentration 2×10^{-3} M were prepared by dissolving 0.367, 0.340 and 0.480 g, of the respective compounds in 1 l of 0.01 M acetic acid. Their purities were established by British Pharmacopoeial (BP) methods.[8]

Determination of Pure Drugs Using *p*-Anisaldehyde and *p*-Tolualdehyde

Introduce an accurately measured volume of noradrenaline or methyldopa solution in 0.01 M acetic acid into a 10-ml calibrated flask, add 1.0 ml of 1.0% sodium hydroxide solution and allow to stand for 5 min. Add 1.0 ml of *p*-anisaldehyde or *p*-tolualdehyde reagent, followed by 5 ml of the acetate buffer. Dilute the solution to volume with water, allow to stand for 20 min and measure the absorbance at 540 nm against a blank prepared similarly but without the active ingredient. Calculate the concentration of noradrenaline or methyldopa from calibration graphs previously prepared in the same manner using different amounts in the ranges 0.16–1.35 and 0.24–1.92 mg of adrenaline and methyldopa, respectively.

Determination of Pure Drugs Using Benzaldehyde

Introduce an accurately measured volume of noradrenaline or methyldopa solution in 0.01 M acetic acid into a 10-ml calibrated flask, add 1.0 ml of 1.0% sodium hydroxide solution and allow to stand for 5 min. Add 1.0 ml of benzaldehyde reagent, followed by 5 ml of the acetate buffer. Dilute the solution to volume with water, allow to stand for 20 min and measure the absorbance at 480 nm against a blank prepared similarly but without the active ingredient. Calculate the concentration of noradrenaline or methyldopa from calibration graphs previously prepared in the same manner using different amounts in the ranges 0.338–2.028 and 0.24–1.92 mg of adrenaline and methyldopa, respectively.

Analysis of Pharmaceutical Dosage Forms

Analyses of various pharmaceutical dosage forms, tablets, injections, solutions and syrups were carried out using the above procedures after making the necessary preparations as stated in the BP methods.[8]

Results and Discussion

As the reaction was enhanced by the use of strong bases, considerable precautions were taken to minimise the decomposition of noradrenaline and methyldopa in alkaline media. A distinct purple or pinkish colour was readily seen with *p*-anisaldehyde, *p*-tolualdehyde and benzaldehyde when the action was carried out in the presence of 1.0 ml of 1.0% *m/V* sodium hydroxide solution (Fig. 1). At higher alkali concentrations the resulting colour disappeared and brown decomposition products were obtained.[9,10]

To establish the optimum conditions for the determination of sympathomimetic amines, the effects of several experimental variables were studied and are reported below.

Effect of pH

The coloured derivatives produced on addition of sodium hydroxide solution were completely destroyed in mineral acids and at pH 7.3. It was observed that a more sensitive and deeper colour was obtained with acetate buffer (pH 4.7). Water did not affect the interaction of the derivatives with *p*-anisaldehyde, *p*-tolualdehyde or benzaldehyde. The highest sensitivity and the maximum stability of the colour were observed with acetate buffer within 10–30 min, as shown in Fig. 2.

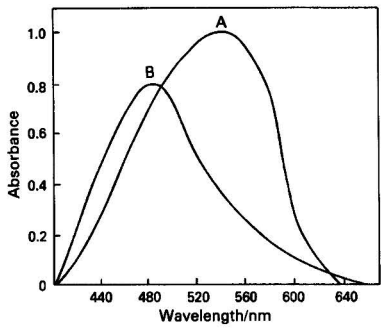


Fig. 1. A, Absorption spectrum of noradrenaline with A, *p*-anisaldehyde and B, benzaldehyde

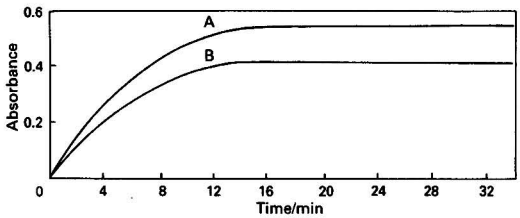
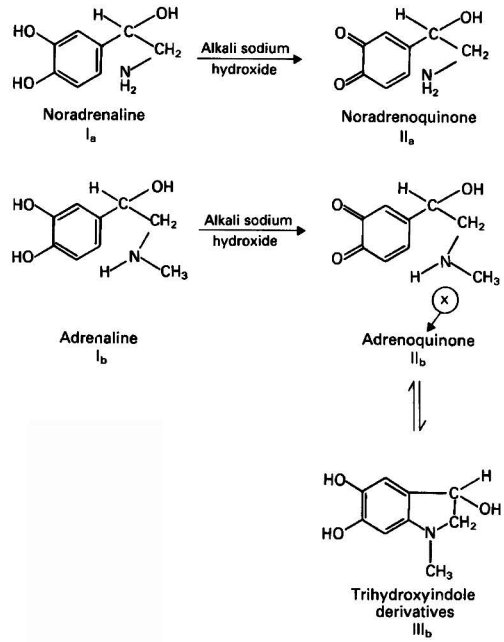


Fig. 2. A, Effect of time on the reaction of A, *p*-anisaldehyde with noradrenaline at 25 °C and measured at 540 nm; and B, benzaldehyde with noradrenaline at 25 °C and measured at 480 nm



Scheme 1

Table 1. Determination of noradrenaline and methyldopa. The mean accuracies by the official methods are 98.67 ± 0.073 and 98.731 ± 0.22, respectively. Each result is the mean of six experiments at $p = 0.05$

		Amount found, %					
		Using *p*-anisaldehyde		Using *p*-tolualdehyde		Using benzaldehyde	
Noradrenaline taken/mg	Methyldopa taken/mg	Noradrenaline	Methyldopa	Noradrenaline	Methyldopa	Noradrenaline	Methyldopa
0.285	0.24	98.62	98.53	98.59	98.62	98.60	98.49
0.458	0.48	98.65	98.64	98.60	98.60	98.60	98.52
0.549	0.72	98.71	98.70	98.58	98.61	98.59	98.53
0.641	0.84	98.71	98.70	98.61	98.63	98.58	98.50
0.846	1.20	98.65	98.69	98.62	98.64	98.55	98.50
1.100	1.320	98.72	98.69	98.59	98.61	98.56	98.48
Mean		98.68	98.66	98.62	98.60	98.58	98.504
S.D.*		0.0401	0.066	0.0134	0.021	0.022	0.0196
t†		0.323	0.934	2.028	0.5	3.0	7.5
F‡		1.389	2.3679	5.09	0.042	2.423	2.838

* Standard deviation.
† Tabulated *t* for 10 degrees of freedom at $p = 0.05 = 2.23$.
‡ Tabulated *F* for (5,5) degrees of freedom at $p = 0.05 = 5.1$.

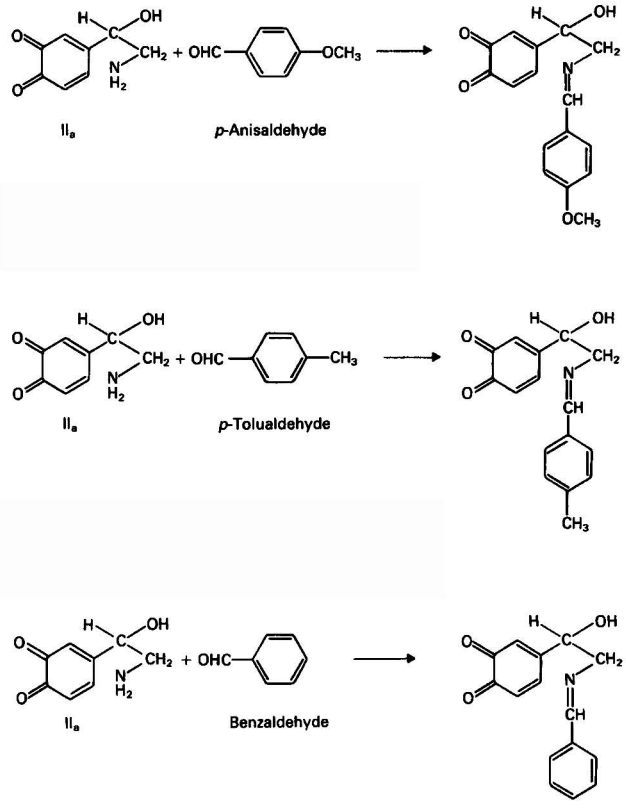
Effect of *p*-Anisaldehyde, *p*-Tolualdehyde, Benzaldehyde and Acetate Buffer Concentrations on Colour Intensity

A study was made to determine the optimum concentrations of the reagents to obtain the maximum colour intensity for a given concentration of methyldopa or noradrenaline. It was found that 1.0 ml of a 0.2% *m/V* solution in methanol of anisaldehyde, *p*-tolualdehyde or benzaldehyde was the optimum for the assay. Different volumes of acetate buffer (pH 4.7) ranging from 1.0 to 7.0 ml were found not to affect the absorbance values.

In view of the results, 5.0 ml of acetate buffer were selected for the assay.

Effect of Temperature

The reaction of *p*-anisaldehyde, *p*-tolualdehyde or benzaldehyde with methyldopa or noradrenaline to form a Schiff base was carried out at elevated temperatures using a boiling water-bath. It was observed that gradual destruction of the intense purple or pink colour was followed by a distinct



Scheme 2

Table 2. Determination of noradrenaline and adrenaline at different concentrations in mixtures

Mixture 1*	Mixture 2*	Mixture 3*	Noradrenaline found, %								
			Using *p*-anisaldehyde			Using *p*-tolualdehyde			Using benzaldehyde		
			1	2	3	1	2	3	1	2	3
0.285	0.338	0.085	98.49	98.64	98.69	98.66	98.68	98.83	98.53	98.66	98.69
0.244	0.183	0.183									
0.458	0.507	0.122	98.50	98.65	98.72	98.67	98.69	98.84	98.50	98.66	98.72
0.413	0.285	0.285									
0.55	0.676	0.169	98.50	98.66	98.70	98.65	98.71	98.85	98.50	98.73	98.73
0.498	0.366	0.366									
0.641	0.845	0.244	98.52	98.67	98.70	98.70	98.73	98.79	98.47	98.72	98.75
0.583	0.458	0.549									
0.732	1.014	0.338	98.53	98.63	98.68	98.71	98.74	98.78	98.46	98.74	98.77
0.676	0.549	0.732									
1.098	1.183	0.583	98.48	98.63	98.71	98.70	98.75	98.88	98.45	98.75	98.72
1.014	0.641	0.915									
Mean ..			98.504	98.65	98.700	98.682	98.72	98.83	98.49	98.71	98.73
S.D.† ..			0.0193	0.0175	0.0146	0.0257	0.0282	0.0347	0.0329	0.042	0.0284
t‡			2.102	0.833	1.0	0.273	1.923	1.6	3.85	2.4	2.31
F§			2.67	2.67	5.1	1.72	1.403	0.942	6	5.3	5.60

* In each instance the upper value is the amount of noradrenaline (mg) and the lower value the amount of adrenaline (mg). The molar ratios of noradrenaline to adrenaline in mixtures 1, 2 and 3 are 1:1, 2:1 and 1:2, respectively.

† Standard deviation.

‡ Tabulated *t* for 10 degrees of freedom at $p = 0.05 = 2.23$.

§ Tabulated *F* for (5,5) degrees of freedom at $p = 0.05 = 5.1$

decrease in the absorbance at 540 or 480 nm. This effect may be due to the side oxidation reactions of catecholamines at elevated temperatures. However, in these experiments a favourable temperature for the reaction was 25 °C.

Chemistry of the Reaction

Noradrenaline and methyldopa did not give the colour directly, but only after the addition of sodium hydroxide solution. This could be explained by the fact that the reaction was due to the coloured derivatives formed. Adrenaline and isopropylnoradrenaline, within the 5-min period of reaction with sodium hydroxide, underwent indole ring closure,[11,12] so that the nitrogen could not react with *p*-anisaldehyde, *p*-tolualdehyde or benzaldehyde to form the coloured conjugated imines that could be measured spectrophotometrically.

It is possible that aminochrome derivatives formed[11,13] by the effect of the alkali on noradrenaline and methyldopa were responsible for the colour formation with these aldehydes.

The reaction pathway may be as shown in Schemes 1 and 2.

Precision and Accuracy

Regression analysis of the Beer's law plots at 540 nm for *p*-anisaldehyde or *p*-tolualdehyde revealed a good correlation (r = 1.0–0.995). Beer's law was obeyed up to concentrations of 0.14 and 0.19 mg ml^{-1} of noradrenaline and methyldopa, respectively, in the sample solution. Regression analysis of the Beer's law plot at 480 nm for benzaldehyde also showed a good correlation up to concentrations of 0.2 and 0.24 mg ml^{-1} of noradrenaline and methyldopa, respectively, in the sample solution.

Table 1 compares the results of the analysis of noradrenaline and methyldopa using *p*-anisaldehyde, *p*-tolualdehyde and benzaldehyde with those given by the official method. The mean percentage recoveries and the standard deviations are satisfactory. For the first two aldehydes, the Student's *t*-test and *F*-ratio show no significant difference between the proposed and the official methods.[8] However, the proposed method using benzaldehyde shows a significant difference with respect to the *t*-value.

Table 3. Determination of noradrenaline in the presence of different concentrations of adrenaline

Noradrenaline taken/mg	Adrenaline added/mg	Noradrenaline found, % Using *p*-anisaldehyde	Using *p*-tolualdehyde	Using benzaldehyde
0.09	0.915	98.66	98.72	98.75
	0.183	98.68	98.69	98.74
	0.366	98.70	98.68	98.72
	0.458	98.67	98.67	98.70
	0.549	98.71	98.71	98.76
	0.641	98.72	98.70	98.71
Mean ..		98.69	98.7	98.73
S.D.* ..		±0.02	0.021	0.0248
t†		1.25	0.698	3.529
F‡		2.81	0.042	1.974

* Standard deviation.
† Tabulated t for 10 degrees of freedom at p = 0.05 = 2.23.
‡ Tabulated F for (5,5) degrees of freedom at p = 0.05 = 5.1.

Determination of Noradrenaline in the Presence of Adrenaline

The proposed methods could be used for the determination of noradrenaline in the presence of different concentrations of adrenaline. Table 2 shows the mean percentage recoveries and the standard deviations for noradrenaline in the presence of adrenaline at molar ratios of 1:1, 2:1 and 1:2. The results indicate no significant difference from the official method except for the proposed method using benzaldehyde.

The validity of the proposed method was tested by determining noradrenaline in the presence of up to 0.64 mg of adrenaline. The results are given in Table 3. Statistical analysis of the results showed that Student's *t* and the *F*-ratio are less than the tabulated values, indicating no significant difference between the official and the proposed procedures when using *p*-anisaldehyde and *p*-tolualdehyde. However, the *t*-test showed a significant difference between the proposed method using benzaldehyde and the official method, which indicates that the two methods are not equally precise.

Analysis of Pharmaceutical Preparations

The proposed methods were applied to the determination of the active ingredients in pharmaceutical preparations, with particular regard to the determination of noradrenaline in the presence of adrenaline. Table 4 shows that the mean recovery of noradrenaline in Baycain Blue ampoules ranged from 98.44 ± 0.26 to 98.95 ± 0.92%; these results are more accurate and precise than those given by the official method.

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Table 4. Determination of noradrenaline and methyldopa in pharmaceutical preparations. Each result is the mean of six experiments with the 95% confidence interval

Samples	Recovery, % Using *p*-anisaldehyde	Using *p*-tolualdehyde	Using benzaldehyde	Official methods
Arterenol ampoules*	98.59 ± 0.23	98.62 ± 0.31	98.75 ± 0.29	98.61 ± 0.18
Baycain Green ampoules† ..	98.12 ± 0.19	98.24 ± 0.25	98.98 ± 0.25	98.06 ± 0.07
Baycain Blue ampoules‡ ..	98.44 ± 26	98.50 ± 0.05	98.95 ± 0.92	97.99 ± 0.02
Aldomet tablets§	98.92 ± 0.42	98.89 ± 0.51	99.01 ± 0.68	98.74 ± 0.03

* Labelled to contain 1 mg ml^{-1} of noradrenaline. Hoechst Laboratories.
† Labelled to contain 0.06 mg ml^{-1} of noradrenaline. Bayer.
‡ Labelled to contain 0.024 mg ml^{-1} of noradrenaline and 0.024 mg ml^{-1} of adrenaline. Bayer.
§ Labelled to contain 250 mg of methyldopa per tablet. Merck, Sharp and Dohme.

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Spectrophotometric Determination of Cobalt, Nickel and Iridium after Coprecipitation of Their Tetrahydrofurfurylxanthates on to Microcrystalline Naphthalene

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Cobalt, nickel and iridium react with potassium tetrahydrofurfurylxanthate to form water-insoluble, thermally stable coloured complexes. These complexes were easily coprecipitated on to microcrystalline naphthalene at pH 2.2–9.5 for cobalt, 3.7–8.6 for nickel and 6.5–9.5 for iridium and their absorption spectra showed maxima at 350–355 nm for cobalt, 415–420 and 480 nm for nickel and 380–387 nm for iridium. Beer's law was obeyed and in the concentration ranges 0–30 μg for cobalt, 10–80 μg for nickel and 35–230 μg for iridium per 10 ml of the final chloroform solution. The molar absorptivities and Sandell's sensitivities were 1.650×10^4 l mol^{-1} cm^{-1} and 0.004 μg cm^{-2} for cobalt, 3.196×10^3 l mol^{-1} cm^{-1} and 0.018 μg cm^{-2} for nickel and 5.013×10^3 l mol^{-1} cm^{-1} and 0.038 μg cm^{-2} for iridium. Ten replicate analyses of solutions containing 12.5 μg of cobalt, 45 μg of nickel and 115 μg of iridium gave mean absorbances of 0.350, 0.245 and 0.300 with relative standard deviations of 0.53, 0.47 and 0.26%, respectively. The interferences of various ions were studied and conditions were developed for the determination of these metals in certain alloys and synthetic mixtures.

Keywords: *Cobalt, nickel and iridium determination; spectrophotometry; potassium tetrahydrofurfurylxanthate; coprecipitation; alloy analysis*

A survey of the literature revealed that common xanthates[1] have been widely utilised as complexing agents for the determination of various metals using a variety of techniques.[2–4] Recently, some substituted xanthates have also been reported as being sensitive and selective complexing agents for the determination of cobalt,[5] gold[6] and tellurium.[7] In this paper a method has been developed for the determination of cobalt, nickel and iridium in various synthetic samples and alloys after coprecipitating their tetrahydrofurfurylxanthates on to microcrystalline naphthalene. It has been observed that the higher homologue xanthates are relatively more stable than the lower ones and therefore potassium tetrahydrofurfurylxanthate has been employed. It has been found to be more sensitive than reported substituted xanthates. The various advantages of the technique of coprecipitation on to microcrystalline naphthalene have already been discussed.[7] In this work, parameters such as effect of pH, reagent concentration, digestion time, naphthalene concentration, shaking time and aqueous phase volume have been studied.

Experimental

Equipment

An Elico pH meter and Pye Unicam SP-500 and SP-700 recording spectrophotometers were used.

Reagents

Distilled water and analytical-reagent grade chemicals were used throughout.

Potassium tetrahydrofurfurylxanthate solution, 0.2%. Prepare the tetrahydrofurfurylxanthate salt by mixing potassium hydroxide, tetrahydrofurfuryl alcohol and carbon disulphide (1 + 1 + 1) in diethyl ether at a low temperature (below 4 °C) using ice - water. Separate the solid by filtration, wash with diethyl ether and recrystallise.[8] Dissolve the solid in water to prepare a 0.2% reagent solution.

Metal standard solutions, 10^{-2} M. Dissolve cobalt(II) chloride, nickel(II) nitrate and iridium(III) chloride in water and standardise by established methods.[9]

Perchloric acid, 1 M.

Ammonia solution, 1 M.

Naphthalene solution, 20%. Prepare in acetone.

Naphthalene. Check the purity spectrophotometrically before use.

General Procedure

Transfer about 40 ml of each sample solution containing 12.5 μg of cobalt, 45 μg of nickel or 115 μg of iridium into a 100-ml Erlenmeyer flask with a tightly fitting stopper. Add 2 ml of 0.2% reagent solution and adjust to pH 2.2–9.5 for cobalt, 3.7–8.6 for nickel and 6.5–9.5 for iridium with perchloric acid and ammonia solution. Mix the solution well, allow it to stand for 2 min, then add 2 ml of 20% naphthalene solution and shake vigorously for 1 min. Filter through a filter-paper (*e.g.*, No. 5C, Toyo Roshi Co.) placed flat on a filter-plate in a funnel. Dissolve the solid in chloroform, dilute to 10 ml and measure the absorbance in a 1-cm cell at 352 nm for cobalt, 417 nm for nickel or 385 nm for iridium against a reagent blank prepared similarly.

Results and Discussion

Absorption Spectra

The absorption spectra of potassium tetrahydrofurfurylxanthate and its cobalt, nickel and iridium complexes in naphthalene - chloroform solution were recorded against water and the reagent blank, respectively (Fig. 1). The maximum absorptions were found to be at 350–355 nm for cobalt, 415–420 nm for nickel and 380–387 nm for iridium complexes; the absorption of the reagent was negligible at these wavelengths. Although the nickel complex was also absorbed at 480 nm, the magnitude of the absorbance was low. For the quantitative determination of these metals as their tetrahydrofurfurylxanthates, the absorbances were measured at 352 nm for cobalt, 417 nm for nickel and 385 nm for iridium as the absorbances were maximal at these wavelengths.

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Effect of pH

Coprecipitations were carried out at different pH values under optimum conditions. It was observed that the absorbances were constant in the pH ranges 2.2–9.5 for cobalt, 3.7–8.6 for nickel and 6.5–9.5 for iridium (Fig. 2).

Effect of Reagent Concentration

Various volume of 0.2% reagent solution were added to the sample solution containing 12.5 μg of cobalt, 45 μg of nickel or 115 μg of iridium at their respective pH values. The absorbances remained constant when the volume of the reagent solution used was more than 0.5 ml in each instance. Therefore, 2 ml of 0.2% reagent solution was chosen for the quantitative determination of these metals.

Effect of Digestion Time

Each of the metal solutions in the presence of 2 ml of 0.2% reagent solution was digested at room temperature and treated as described in the General Procedure. It was observed that the absorbance increased with increase in the digestion time up to 2 min, then remained almost constant between 2 and 10 min. Therefore, a digestion time of 3–5 min was chosen for complete reaction of each metal ion with the reagent.

Effect of Naphthalene

Coprecipitation of each metal complex was carried out by varying the volume of 20% naphthalene solution. The absorbance increased with increase in the amount of naphthalene solution up to 0.5 ml and then remained almost constant between 0.5 and 5.0 ml. Therefore, 2 ml of 20% naphthalene solution was chosen for the coprecipitation of each metal complex.

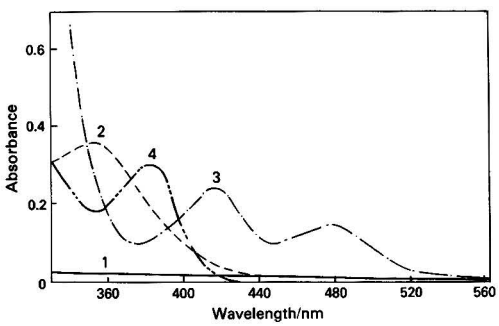

Fig. 1. Absorption spectra of 1, reagent blank against water; and of tetrahydrofurfurylxanthates of 2, cobalt; 3, nickel; and 4, iridium in naphthalene - chloroform solution against reagent blank. Co, 12.5 μg; Ni, 45 μg; Ir, 115 μg; 0.2% reagent solution, 2 ml for each metal; pH, 8.0; 20% naphthalene solution, 2 ml for each coprecipitation

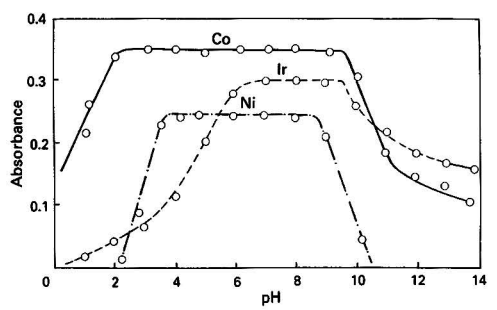

Fig. 2. Effect of pH on absorbance. Co, 12.5 μg; Ni, 45 μg; Ir, 115 μg; 0.2% reagent solution, 2 ml for each metal; 20% naphthalene solution, 2 ml for each coprecipitation; $\lambda_{max.}$, 352 nm for cobalt, 417 nm for nickel and 385 nm for iridium; reference, reagent blank

Effect of Shaking Time

A 2-ml volume of 20% naphthalene solution was added to the sample solution containing each metal complex, and the mixture was then shaken vigorously for 10–100 s. The results showed that each metal complex was coprecipitated quantitatively with microcrystalline naphthalene on vigorous shaking over that period. Therefore, a shaking time of 1 min was chosen for the complete coprecipitation of each metal complex.

Effect of Volume of the Aqueous Phase

The volume ratio of the organic to the aqueous phase is an important factor for the coprecipitation of each metal complex with naphthalene. Therefore, the effect of the latter on the absorbance was examined. It was found that the absorbance remained constant for up to an 80-ml volume of aqueous phase; increasing this volume caused a gradual decrease in absorbance.

Effect of Standing Time

The coprecipitated naphthalene mixture was dissolved in chloroform and the effect of standing time on the absorbance was investigated. The results indicated that the cobalt complex in chloroform was stable for 48 h, the nickel complex for 24 h and the iridium complex for 6 h. Therefore, a 30-min standing time was selected for all the absorbance measurements.

Choice of Organic Solvent

Various organic solvents were examined for dissolving each coprecipitated metal complex. The complex - naphthalene mixture, in all instances, is soluble in chloroform, benzene, carbon tetrachloride, isobutyl methyl ketone, dimethylformamide, chlorobenzene, 1,2-dichloroethane, *o*-xylene and acetonitrile. However, chloroform was preferred as the absorbance of each metal complex was found to be maximal in this solvent.

Composition of the Metal Complexes

The composition of the metal complexes was determined by the logarithmic method,[10] in which the reaction of a metal ion M^{n+} with reagent HR giving a chelate MR_n may be represented as follows:

$$M^{n+} + nHR \rightleftharpoons MR_n + nH^+$$

The equilibrium constant K is given by

$$K = \frac{[MR_n][H^+]^n}{[M^{n+}][HR]^n}$$

which can be expressed as

$$\text{Log}([MR_n]/[M^{n+}]) = n\text{log}[HR] - n\text{log}[H^+] + \log K$$

where $[MR_n]$ and $[M^{n+}]$ are the concentrations of complex formed and free metal ion, respectively, and [HR] is the concentration of the ligand. If a graph is plotted of $\log([MR_n]/[M^{n+}])$ *versus* log[HR] at a constant hydrogen ion concentration, a straight line is obtained with a slope equal to n, which corresponds to the number of ligand molecules bound per metal ion.

The values of $\log([MR_n]/[M^{n+}])$ and log[HR] were calculated and plotted (Fig. 3). Straight lines with slopes equal to

Table 1. Effect of anions on the determination of cobalt, nickel and iridium. Conditions: Co, 12.5 μg; Ni, 45 μg; Ir; 115 μg; pH, 8.0; 0.2% reagent solution, 2 ml for each metal; 20% naphthalene solution, 2 ml for each coprecipitation

Salt of the anion added	Amount of salt added/mg	Absorbance: Cobalt 352 nm	Nickel 417 nm	Iridium 385 nm
—	—	0.350	0.245	0.300
Sodium fluoride	100	0.350	0.243	0.300
Potassium bromide	100	0.350	0.245	0.298
Potassium iodide	100	0.350	0.237	0.300
Sodium carbonate	100	0.346	0.240	0.300
Trisodium phosphate	100	0.346	0.110	0.300
	10	0.350	0.243	0.300
Potassium sulphate	100	0.350	0.244	0.300
Potassium thiocyanate	100	0.246	0.240	0.300
	50	0.338	0.245	0.300
Sodium acetate	100	0.350	0.245	0.300
Sodium citrate	100	0.342	0.078	0.300
	5	0.350	0.244	0.300
Sodium oxalate	100	0.344	0.060	0.298
	5	0.350	0.240	0.300
Ammonium tartrate	100	0.350	0.242	0.298
Disodium EDTA	100	0.005	0.005	0.298
	5	0.015	0.018	0.300

Table 2. Effect of cations on the determination of cobalt, nickel and iridium. Conditions as given in Table 1

Metal salt added	Metal ion added/μg	Absorbance: Cobalt 352 nm	Nickel 417 nm	Iridium 385 nm
		0.350	0.245	0.300
Aluminium nitrate	500	0.350	0.245	0.300
Ammonium molybdate	500	0.353	0.245	0.300
Ammonium metavanadate	500	0.457	0.415	0.322
	200	0.356	0.251	0.301
Antimony(III) chloride	500	0.335	0.230	0.300
	300	0.347	0.245	0.300
Bismuth nitrate	500	0.366	0.248	0.302
	300	0.352	0.245	0.300
Cadmium chloride	500	0.228	0.175	0.286
	200	0.342	0.239	0.300
Chromium(III) nitrate	500	0.350	0.245	0.300
Cobalt(II) chloride	500	—	0.305	0.425, 0.302*
	150	—	0.250	0.303
Copper(II) chloride	500	—, 0.353†	0.620, 0.245†	0.520, 0.308*
Gold(III) chloride	500	0.350	0.245	0.300
Indium(III) sulphate	500	0.346	0.341	0.300
Iridium(III) chloride	300	0.485, 0.352†	0.318, 0.245†	—
Iron(III) chloride	500	0.885, 0.353‡	0.810, 0.250‡	0.906, 0.304‡
Lead(II) nitrate	500	0.345	0.240	0.208
Manganese(II) acetate	500	0.350	0.245	0.300
Mercury(II) chloride	500	0.265	0.220	0.281
	200	0.344	0.243	0.298
Nickel(II) chloride	500	—, 0.354§	—	0.760, 0.307*
Osmium(VIII) tetraoxide	500	0.350	0.245	0.300
Palladium(II) chloride	500	0.765	0.323	0.303
	25	0.358	0.250	0.300
Platinum(IV) chloride	500	0.350	0.245	0.300
Rhodium(III) chloride	500	0.368	0.271	0.307
	250	0.350	0.250	0.302
Ruthenium(III) chloride	500	0.364	0.259	0.312
	300	0.352	0.246	0.300
Sodium arsenite(III)	500	0.350	0.245	0.300
Sodium tungstate(VI)	500	0.350	0.245	0.300
Tin(II) chloride	500	0.350	0.245	0.300
Thorium(IV) nitrate	500	0.348	0.243	0.300
Uranyl acetate	500	0.350	0.248	0.300
Zinc sulphate	500	0.344	0.342	0.300
Zirconyl chloride	500	0.350	0.245	0.300

* After masking with 5 ml of 10% EDTA solution.
† After masking with 5 ml of 10% thiourea solution.
‡ After masking with 5 ml of 10% ascorbic acid solution.
§ Cobalt was determined at pH 2.5.

2.0 for cobalt, 1.9 for nickel and 2.75 for iridium were obtained, which suggests the formation of $Co(OCH_2CH_2CH_2CHCH_2OCS_2)_2$, $Ni(OCH_2CH_2CH_2CHCH_2OCS_2)_2$ and $Ir(OCH_2CH_2CH_2CHCH_2OCS_2)_3$.

Beer's Law and Sensitivity

Calibration graphs for these metals were constructed under the optimum conditions. The graphs obey Beer's law over the concentration ranges 0–30 μg for cobalt, 10–80 μg for nickel and 35–230 μg for iridium per 10 ml of the final solution. The molar absorptivities and sensitivities were calculated to be 1.650×10^4 l mol^{-1} cm^{-1} and 0.004 μg cm^{-2} for cobalt, 3.196×10^3 l mol^{-1} cm^{-1} and 0.018 μg cm^{-2} for nickel and 5.013×10^3 l mol^{-1} cm^{-1} and 0.038 μg cm^{-2} for iridium, respectively. Ten replicate determinations of 12.5 μg of cobalt, 45 μg of nickel and 115 μg of iridium gave mean absorbances of 0.350, 0.245 and 0.300 with relative standard deviations of 0.53, 0.47 and 0.26%, respectively.

Interference from Diverse Ions

The interference from various ions was studied under optimum conditions. In general, a 100-mg amount of salt of the studied anions and an amount of salt equivalent to 500 μg of metal ions were added individually to aliquots containing 12.5 μg of cobalt, 45 μg of nickel and 115 μg of iridium. Among the anions examined, phoshate, citrate and oxalate interfered seriously in the determination of nickel; EDTA interfered in the determination of both nickel and cobalt; and thiocyanate interfered in the determination of cobalt only. The tolerance limits for these anions are given in Table 1. Among the cations examined (Table 2), Cu^{2+}, Ni^{2+}, Fe^{3+} and Pd^{2+} interfered seriously in the determination of cobalt; Cu^{2+}, Fe^{3+}, Co^{2+} and Pd^{2+} interfered seriously in the determination of iridium.

Table 3. Determination of cobalt in alloys

Alloy	Composition, %	Amount of cobalt taken/ μg	Amount of cobalt found/ μg	Average/ μg	Relative standard deviation, %
JSS, 607-6 high-speed steel	Co, 4.72; V, 0.86; W, 16.96; Mo, 0.30; Cr, 4.14; Ni, 0.058; Cu, 0.028; S, 0.006; P, 0.012; Mn, 0.30; Si, 0.32; C, 0.75	17.3	17.62 17.38 17.20 17.43 17.11	17.35	1.15
		25.8	25.97 26.05 25.62 25.86 25.93	25.89	0.63
JSS, 655-4 stainless steel	Co, 0.28; Nb, 0.60; Te, 0.03; W, 0.024; Mo, 0.051; Cr, 18.54; Ni, 11.48; Cu, 0.088; S, 0.006; P, 0.033; Mn, 1.58; Si, 0.60; C, 0.055	21.6	21.89 21.37 21.82 21.48 21.95	21.70	1.20
		27.2	27.67 26.95 27.05 27.42 27.36	27.29	1.07

Table 4. Determination of nickel in alloys

Alloy	Composition, %	Amount of nickel taken/ μg	Amount of nickel found/ μg	Average/ μg	Relative standard deviation, %
Aluminium	Ni, 1.025; Cu, 1.175; Fe, 0.300; Mg, 1.2; Si, 18.5	28.5	28.16 28.30 28.95 28.70 28.27	28.48	1.17
		57.0	57.45 57.20 56.58 56.83 57.13	57.04	0.59
Steel	Ni, 0.44; Cu, 0.54; C + Si + Mn + Cr, 35.02	34.1	34.51 34.60 33.78 33.90 34.22	34.20	1.06
		68.2	68.85 68.02 68.55 67.96 68.24	68.32	0.55

Table 5. Determination of iridium in synthetic samples

Composition of sample, %	Amount of iridium taken/ μg	Amount of iridium found/ μg	Average/ μg	Relative standard deviation, %
Ir, 28; Pt, 55; Rh, 7; Cu, 3; Fe, 3.5; Pd, 3.5	40.0	40.38 39.76 40.28 40.12 39.84	40.08	0.67
	80.0	80.67 79.85 79.98 80.19 79.83	80.10	0.43
Ir, 7; Pd, 25; Pt, 15; Rh, 15; Os, 20; Au, 18	60.0	60.51 60.12 59.75 59.90 60.08	60.08	0.48
	120.0	120.62 120.19 119.66 119.83 120.27	120.11	0.32

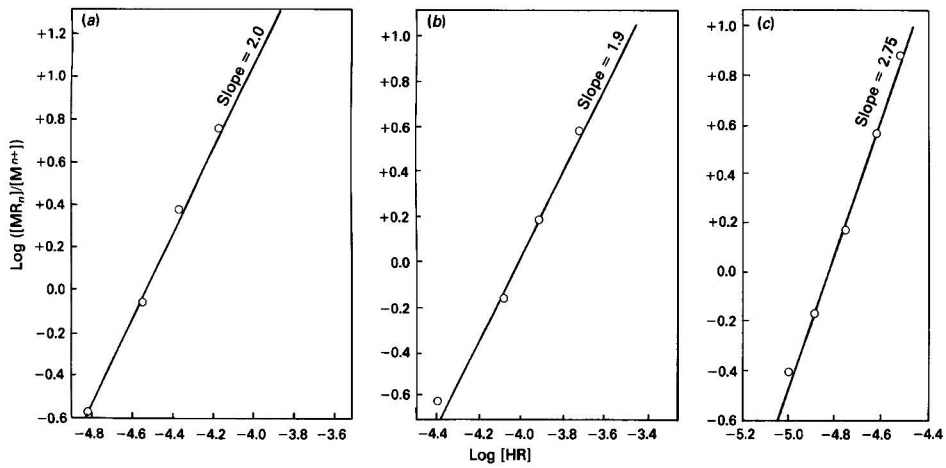

Fig. 3. Logarithmic method for the determination of (*a*) cobalt, (*b*) nickel and (*c*) iridium in solution. Initial concentrations: Co, 0.3 × 10^{-3} M; Ni, 0.8 × 10^{-3} M; and Ir, 1 × 10^{-3} M. Reagent concentrations: Co, 0.3 × 10^{-3} M; Ni, 0.8 × 10^{-3} M; and Ir, 1 × 10^{-3} M. Other conditions as given in Fig. 1

Iron(III) interfered in all determinations, and was masked with 5 ml of 10% ascorbic acid solution. The interference from Ni(II) in the determination of cobalt could be eliminated if the coprecipitation was carried out around pH 2.5, while that of Cu(II) and Ni(II) in the determination of iridium was eliminated by masking with 5 ml of 10% EDTA solution. Relatively small amounts of the remaining metallic ion interferents could be tolerated (Table 2).

Determination of Cobalt and Nickel in Alloys

Each sample (0.1–1.0 g) of the alloys of cobalt and nickel was dissolved in 15–20 ml of concentrated hydrochloric acid, followed by a few drops of concentrated nitric acid with heating. The solution was next evaporated nearly to dryness. Another 10 ml of concentrated hydrochloric acid were added and the mixture was diluted with water, filtered and the filtrate diluted to 500 ml in a calibrated flask. For the determination of cobalt, an aliquot of the sample solution was adjusted to pH 2.5 if nickel was present. Higher amounts of copper and iron in both the determinations were removed by pre-extracting their benzoylacetonates[11] into benzene at pH 4.0. The aqueous phase was then treated as described under the General Procedure for the determination of cobalt and nickel. The results are given in Tables 3 and 4.

Determination of Iridium in Synthetic Samples

Different amounts of metal salts including those of iridium were mixed in such a way that the resultant mixtures corresponded to some standard reference samples such as alloys. The mixture (0.2–0.5 g) was dissolved in aqua regia with heating and evaporated nearly to dryness. Concentrated hydrochloric acid (10 ml) was then added and the solution was

warmed. After cooling to room temperature it was diluted and then filtered and the filtrate was diluted to 500 ml in a calibrated flask. To an aliquot of the sample solution at pH 8.0, 5 ml of 10% ascorbic acid were added if iron was present, which was then treated as described under the General Procedure. The results are given in Table 5.

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Spectrophotometric Determination of Cobalt and Nickel with 1,5-Bis(di-2-pyridylmethylene)thiocarbonohydrazide

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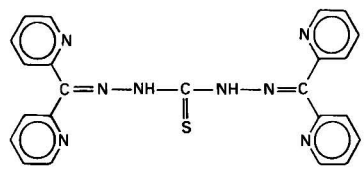
1,5-Bis(di-2-pyridylmethylene)thiocarbonohydrazide (DPTH) has been examined to evaluate its usefulness as a sensitive spectrophotometric reagent for the determination of nickel and cobalt. Two photometric methods are described for the determination of trace amounts of cobalt, one of which has been applied to the determination of cobalt in steels. A method for the determination of trace amounts of cobalt and nickel in mixtures has also been developed.

Keywords: *Cobalt determination; nickel determination; spectrophotometry; 1,5-bis(di-2-pyridylmethylene)-thiocarbonohydrazide*

Thiosemicarbazones and phenylthiosemicarbazones have been used widely as analytical reagents in spectrophotometry, fluorimetry, gravimetry and also as visual indicators. Their analytical applications have been reviewed[1-3] and the structures of their metal complexes investigated.[4] In general, because of the great ability of sulphur for coordinating metal cations, establishing selective methods for the determination of metals is difficult; however, owing to the acid dissociation of the thiosemicarbazone group, the formation of chelates is pH dependent. This behaviour, coupled with the appropriate masking agents, has been used for the selective determination of diverse metal ions. However, few analytical data have been published dealing with the use of thiocarbohydrazones for this purpose, in spite of the colour reactions observed between these compounds and transition metal ions.

During studies on thiocarbonohydrazones derived from aldehydes and α-*N*-heterocyclic ketones, it was found[5] that 1,5-bis(di-2-pyridylmethylene)thiocarbonohydrazide (DPTH) (**I**) offers interesting analytical possibilities, superior to those of di-2-pyridyl ketone thiosemicarbazone[6] and the corresponding phenylthiosemicarbazone[7] owing to the higher molar absorptivities of the chelates ($>10^4$ in all instances) and to the shift of the absorption peaks to longer wavelengths. DPTH shows only two ionisation steps, with pK_a values at 3.07 and 9.85; the first is due to the pyridine rings and the second can be attributed to the loss of the proton of the –SH group.



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In this paper, which forms part of a systematic investigation of the analytical applications of thiocarbonohydrazones, a detailed study of the experimental conditions involved in the formation of cobalt - DPTH and nickel - DPTH complexes is reported. The reagent has been applied to the simultaneous spectrophotometric determination of cobalt and nickel in mixtures.

Experimental

Synthesis of the Reagent

A 0.4 g (0.0038 mol) mass of thiocarbonohydrazide was dissolved in 50 ml of distilled water and 1.7 g (0.098 mol) of di-2-pyridyl ketone in 10 ml of ethanol and 0.5 ml of glacial acetic acid were added. The mixture was then refluxed for 2 h and the yellow product was filtered, crystallised from ethanol and dried in a vacuum desiccator (yield 74%). The product had a melting-point of 172–174 °C and elemental analysis gave the following results: C 63.2, H 4.3, N 25.6 and S 7.5%; $C_{23}H_{18}N_8S$ requires C 63.01, H 4.11, N 25.57 and S 7.31%.

Apparatus

A Shimadzu 240 spectrophotometer was used for recording spectra in the ultraviolet and visible regions of the spectrum and a Pye Unicam SP 6-550 instrument was used for measurements at fixed wavelengths, both equipped with 1 cm glass or silica cells.

A Radiometer pH meter, with glass - calomel electrodes, was used for the pH measurements.

Reagents

Analytical-reagent grade chemicals were used throughout. 1,5-Bis(di-2-pyridylmethylene)thiocarbonohydrazide (DPTH) was used as a 0.05% *m/V* solution in dimethylformamide (DMF). This solution is stable for more than a week.

Standard solutions of cobalt(II) (9.998 mg ml^{-1}) and nickel(II) (4.852 mg ml^{-1}) were prepared from cobalt nitrate hexahydrate and nickel sulphate hexahydrate, respectively, and standardised titrimetrically with EDTA and gravimetrically with dimethylglyoxime, respectively. Working standard solutions were prepared by suitable dilution of the standard solutions.

Procedures

Determination of cobalt with DPTH in alkaline medium (procedure A)

Into a 25-ml calibrated flask transfer a volume of solution containing 2.5–25 μg of cobalt, adjust the pH to 10.4–11.0 with 5 ml of ammonia buffer solution, add 5 ml of 0.05% *m/V* DPTH solution in DMF and 5 ml of DMF (to prevent precipitation of the reagent) and dilute to the mark with distilled water. After 30 min measure the absorbance at 480 nm against a reagent blank prepared in a similar way but without cobalt. Construct calibration graphs for the standard solutions treated in the same way.

Determination of cobalt with DPTH in acidic medium (procedure B).

To the cobalt solution (2.5–25 μg of cobalt) in a 25-ml calibrated flask, add 3.5 ml of concentrated perchloric acid and 5 ml of 0.05% *m/V* DPTH solution in DMF and dilute to volume with water. Measure the absorbance at 410 nm against a reagent blank prepared simultaneously with the sample.

Determination of nickel with DPTH

To the solution containing 5–20 μg of nickel in a 25-ml calibrated flask, add 5 ml of acetate buffer solution of pH 4.3, 7 ml of 0.05% reagent solution and 5 ml of DMF and dilute to volume with water. After 60 min, measure the absorbance at 410 nm against a reagent blank prepared in a similar way. The calibration graph is prepared using standard solutions of nickel(II) treated in the same way.

Determination of nickel and cobalt mixtures

To the solution of nickel and cobalt (5–20 μg of each), in a 25-ml calibrated flask, add 5 ml of ammonia buffer solution of pH 10.4, 7 ml of 0.05% reagent solution in DMF and 5 ml of DMF and dilute to volume with water. After 60 min, measure the absorbance at 440 and 480 nm against a reagent blank, and calculate the cobalt and nickel concentrations by solving the two simultaneous equations described under Applications.

Determination of cobalt in steels

Dissolve a known amount of accurately weighed sample (0.5 g) in about 30 ml of hydrochloric acid (1 + 1) and heat gently until dissolution is complete. Add 10 ml of nitric acid (1 + 1) and continue boiling until nitrous vapours are no longer evolved. Dilute with distilled water to 250 ml in a calibrated flask. Place an aliquot of this solution containing 5–25 μg of cobalt in a 25-ml calibrated flask, add 5 ml of reagent and 0.6 ml of a 10^{-2} M EDTA solution to prevent interference from iron and adjust to pH 10.4–11 with sodium hydroxide solution. The analysis is completed as described under *Procedure A*.

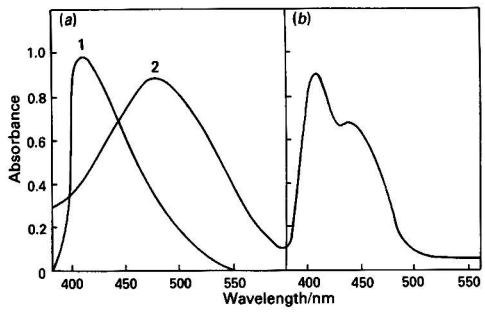

Fig. 1. Absorption spectra of (*a*) cobalt - DPTH complexes (1 p.p.m. of Co) in 1, acidic medium and 2, basic medium; and (*b*) Ni - DPTH complex (1.3 p.p.m. of Ni)

Results and Discussion

Study of the Cobalt - DPTH System

Absorption spectra

When dilute solutions of cobalt(II) and DPTH were mixed, a soluble yellow complex was obtained. The yellow colour ($\lambda_{max.}$ = 410 nm) of the complex changed with increase in pH to red ($\lambda_{max.}$ = 480 nm), the system being reversible. The absorption spectra of coloured solutions underwent bathochromic and hypochromic shifts with pH [Fig. 1(*a*)].

Effect of pH

The absorbance *versus* pH graphs for the cobalt complexes were determined at the maximum wavelengths (410 and 480 nm) of both complexes. A yellow complex was formed at pH 3.5 and no change in absorbance was observed in very acidic solutions, until a concentration of 2.3 N perchloric acid. A red complex was formed in basic medium and the optimum pH range was 10.4–11.0 [Fig. 2(*a*)].

Stability of the complexes

The cobalt - DPTH complex in aqueous acidic medium was stable and gave a constant absorbance even after 13 h. The red complex was formed immediately at pH 10.4–11.0 in aqueous DMF solution, but the absorbance at 480 nm increased with time; the stable red colour was obtained within 30 min.

Effect of DPTH concentration

Two series of 25-ml calibrated flasks were prepared. The concentration in Co(II) of one (procedure A) was 1.3 p.p.m. and that of the other was 0.7 p.p.m. (procedure B). Both series contained increasing amounts of the reagent. After waiting for 30 min (procedure A) or 60 min (procedure B), the absorbance measurements were made at 480 and 410 nm. From the results obtained it appears that the excess of reagent had no additional effect on the absorbance. In all samples, 5 ml of 0.05% reagent solution in DMF were used.

Composition of the complexes

The molar composition of the complex formed at pH 10.6 under the conditions described for the determination of cobalt was ascertained by Job's method of continuous variations and the molar-ratio method. Both methods indicated the formation of a 1 : 1 complex. In order to determine the composition of the yellow complex, the samples were prepared at pH 1.7 and the absorbance was measured at 410 nm; both methods indicated the formation of a 1 : 2 (metal to ligand) complex under either of the conditions employed for the determination of cobalt.

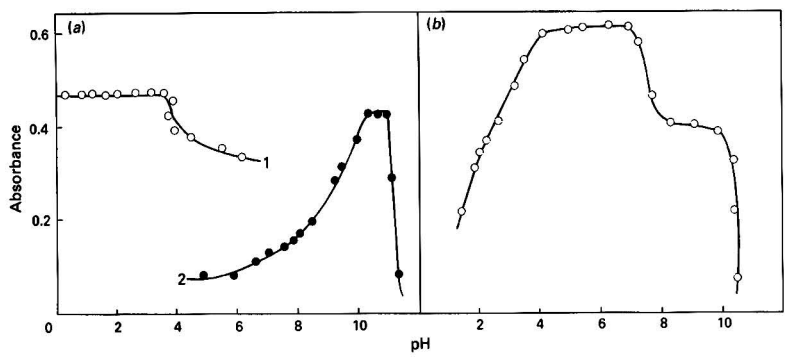

Fig. 2. Influence of pH on the formation of (*a*) Co - DPTH complexes (0.5 p.p.m. of Co) for 1, yellow complex (410 nm) and 2, red complex (480 nm); and (*b*) Ni - DPTH complex (410 nm, 0.5 p.p.m. of Ni)

Study of the Nickel - DPTH System

When a few drops of a DMF solution of DPTH were added to a nickel(II) solution, a yellow complex was formed immediately; this yellow colour changed to orange within a few minutes. The spectrum of the nickel complex showed two maxima, at 410 and 440 nm.

Formation and stability of the nickel complexes

The orange complex was formed immediately in aqueous DMF solution, but the absorbance at 440 nm decreased with time, whereas the absorbance of the yellow complex at 410–420 nm increased and the system became stable within 50 min.

Influence of pH

The influence of pH in the range 1–12 was studied using a series of nickel solutions. The graph of absorbance *versus* pH [Fig. 2(*b*)] showed that the optimum pH range for formation of the orange nickel complex was 4.0–6.8.

Effect of the concentration of reagent

The absorbance of the yellow complex was studied as a function of the molar ratio between DPTH and nickel(II). A one-fold molar excess of reagent was needed to obtain maximum absorbance, which afterwards remained constant with increase in concentration. Hence 7 ml of 0.05% *m/V* reagent solution were adopted.

Composition of the complexes

The composition of the nickel - DPTH complex obtained at pH 4.3 was determined by the methods of Job,[8] Yoe and Jones[9] and Harvey and Manning.[10] The results showed a stoicheiometry of metal to ligand of 1:2.

Spectrophotometric Determination of Cobalt with DPTH

Straight-line calibration graphs passing through the origin were obtained using the recommended procedures. The molar absorptivities (ε) were evaluated by a statistical method. The red complex in aqueous DMF solution (pH 10.6) gave a value of $\varepsilon = 5.2 \times 10^4$ l mol^{-1} cm^{-1} at 480 nm and the yellow complex $\varepsilon = 5.8 \times 10^4$ l mol^{-1} cm^{-1} at 410 nm. The optimum ranges for the determination of cobalt were 0.2–0.7 p.p.m. of cobalt in procedure A and 0.3–0.8 p.p.m. of cobalt in procedure B.

Two series of ten standard solutions each containing 5.2 μg of cobalt were analysed by the recommended procedures. The results gave relative standard deviations of 0.43 and 0.59% ($P = 0.05$) for procedures A and B, respectively.

Spectrophotometric Determination of Nickel(II) with DPTH

Based on the above experimental work, a method for the determination of small amounts of nickel(II) is proposed. In aqueous DMF solution at pH 4.3, DPTH reacted instantly with nickel(II); the complex obeyed Beer's law from 5 to 20 μg with an optimum range, evaluated by the Ringbom method, of 7.5–17.5 μg. The molar absorptivity at 410 nm was 5.01×10^4 l mol^{-1} cm^{-1}. The relative standard deviation was 0.5%. The molar absorptivity of the orange complex at 440 nm was 4.0×10^4 l mol^{-1} cm^{-1}.

Effect of Diverse Ions

The effects of foreign ions on the determination of cobalt by procedures A and B are summarised in Tables 1 and 2, respectively. The tolerance limits showed that cobalt could be determined in the presence of a large number of ions. Interferences from ions on the determination of nickel were similar, but the interference from iron could not be eliminated by the use of EDTA, so that the determination of nickel in steels is not feasible.

Applications

Simultaneous determination of nickel and cobalt

The two procedures (A and B) devised for the determination of cobalt showed different characteristics. In procedure B (acidic medium) the interferences were lower; however, in procedure A (basic medium), masking agents could be used to prevent interference from diverse ions.

The absorption spectrum of the two mixed complexes is the sum of both separate spectra, so that the total absorbance at

Table 1. Tolerance ratios for various ions in the determination of 0.7 p.p.m. of cobalt by procedure A. Tolerable error, ±2%

Foreign ion	Tolerance ratio: Without masking agent	Tolerance ratio: With masking agent	Masking agent, p.p.m.
Thiourea, thiocyanate	55 000		
PO_4^{3-}, citrate	30 000		
F^-, EDTA	1 500		
Tartrate, nitrilotriacetic acid, triethanolamine, K(I), Na(I), Rb(I), Li(I), NH_4^+	700		
Thiosulphate	400		
Thioglycollic acid	150		
Ca(II)	70		
Be(II), Mg(II), Sr(II), Ba(II), As(III), As(V)	30		
Y(III), Au(III)	15		
Ag(I)	6		
Pb(II), V(V)	3		
Al(III)	1.5		
Cr(III), Mn(II), Ni(II), Zn(II)	1		
Fe(II)	1	9	SCN^-, 20 000
Fe(III)	1	20	EDTA, 100
Cu(II)	1	11	EDTA, 1 000
Hg(II)	1	11	EDTA, 1 000
Cd(II)	1	11	SCN^-, 20 000
Bi(III)	1	30	EDTA, 1 000

Table 2. Tolerance ratio for various ions in the determination of 0.64 p.p.m. of cobalt by procedure B. Tolerable error, ±2%

Foreign ion	Tolerance ratio
Na(I), Ba(II), Li(I), NH_4^+, Tl(I), Rb(I) ..	1500
Ca(II), Sr(II), Mg(II)	800
Pb(II), W(VI), As(V), Sn(II), Al(III), UO_2^{2+}, Th(IV), Mn(II), NO_3^-, PO_4^{3-} ..	150
Cd(II), Mo(VI), Sb(III), Zn(II)	80
La(III)	15
Hg(I), Cu(II), Bi(III), Fe(III), Fe(II), Ni(II) ..	1

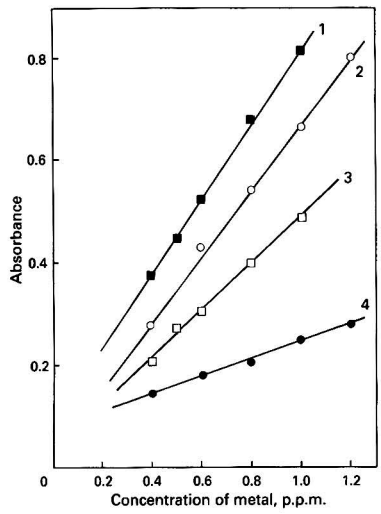

Fig. 3. Calibration graphs used for the simultaneous determination of cobalt and nickel. 1, Co(II), 480 nm; 2, Ni(II), 440 nm; 3, Co(II), 440 nm; and 4, Ni(II), 480 nm

each wavelength may be given as $A_{total} = A_1 + A_2$. Two analytical wavelengths were selected: 440 and 480 nm. To evaluate the molar absorptivities of the complexes at these wavelengths, calibration graphs were plotted as shown in Fig. 3.

To determine trace amounts of cobalt and nickel in a solution, a single sample of a solution is analysed as described under Experimental, and the results are evaluated by solving two simultaneous equations:

$$A_{480} = 1.7 \times 10^4 c_{Ni} + 5.2 \times 10^4 c_{Co}$$
$$A_{440} = 4.0 \times 10^4 c_{Ni} + 3.0 \times 10^4 c_{Co}$$

where c_{Ni} and c_{Co} are the unknown concentrations. Molar absorptivity values are not absolute, and should be measured by the analyst.

The results of the determination of nickel and cobalt in samples with Ni to Co ratios of 0.1–10 are shown in Table 3. These results indicate that the DPTH method is satisfactory.

Determination of cobalt in steels

Only one sample containing cobalt was available, steel G, which has the following composition: C 0.755%, Mn 0.290%, Si 0.316%, Cr 4.223%, P 0.016%, S 0.009%, V 1.077%, W 18.63%, Mo 0.0953% and Co 4.90%. To evaluate the effectiveness of the method, a series of recovery experiments were carried out by adding standard pure cobalt to aliquots of several dissolved steel samples. The composition of these samples was as follows: steel A, Si 0.12%, Mn 0.41%, P 0.024% and S 0.029%; steel B, Si 0.106%, Mn 0.365%, P 0.021%, S 0.034%, W 7.01% and Mo 5.26%; steel C, Si 0.12%, Mn 0.43%, P 0.036%, S 0.044%, W 0.46%, V 1.9% and Cr 2.86%; steel D, Si 0.12%, Mn 1.40%, P 0.024% and S 0.029%; steel E, Si 0.11%, Mn 0.38%, P 0.02%, S 0.027% and W 7.41%; steel F, Si 0.108%, Mn 0.388%, P 0.032%, S 0.040%, W 0.415%, Mo 6.25% and Cr 4.46%. Table 4 shows the results obtained for each sample.

Table 3. Simultaneous determination of nickel and cobalt in mixtures

Sample No.	Ni to Co ratio	Amount taken, p.p.m. Ni	Amount taken, p.p.m. Co	Ni found, p.p.m.	Co found, p.p.m.
1	1:1	0.5	0.5	0.4	0.5
2	1:2	0.3	0.6	0.3	0.6
3	1:3	0.2	0.6	0.2	0.6
4	1:5	0.1	0.5	0.1	0.5
5	1:7	0.1	0.7	0.1	0.7
6	1:9	0.1	0.9	0.1	0.8
7	1:10	0.1	1.0	0.1	0.9
8	2:1	0.6	0.3	0.6	0.3
9	3:1	0.6	0.2	0.6	0.2
10	5:1	1.0	0.2	1.1	0.2
11	7:1	0.7	0.1	0.7	0.1
12	9:1	0.9	0.1	0.8	0.1
13	10:1	1.0	0.1	0.9	0.1

Table 4. Determination of cobalt in steel samples by procedure A

Steel	Cobalt content, % Added	Found	Reported
A	4.86	4.76	—
B	4.20	4.29	—
C	5.40	5.59	—
D	4.72	4.81	—
E	4.42	4.51	—
F	5.08	5.25	—
G	—	4.80	4.90

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Effect of Inorganic Matrices on the Determination of Cadmium by Atomic-absorption Spectrometry with Electrothermal Atomisation*

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The atomisation energies of various cadmium compounds were determined and their values compared with literature data. On this basis, mechanisms of atomisation of cadmium nitrate, chloride and sulphate are postulated. Atomisation was also studied in the presence of sulphuric, nitric and hydrochloric acids and their sodium, calcium and magnesium salts. Taking into account the shape, position and height of the peaks, the probable mechanism of interference was established. Minimum interference effects were obtained when the samples contained nitric acid or metal nitrates.

Keywords: *Atomic-absorption spectrometry; cadmium determination; matrix effects; atomisation mechanism*

Cadmium belongs to a group of toxic metals that are frequently determined by electrothermal atomic-absorption spectrometry. Direct analysis of biological materials is often difficult and such samples need to be mineralised. Blood and Grant[1] used concentrated nitric acid or an acid mixture but did not mention their effect on the determination. Wegscheider *et al.*[2] investigated the effect of various inorganic acids on the determination of cadmium but stated that they did not influence the peak height. Lund and Larsen[3] suggested the addition of nitric acid in urine analysis but mentioned that inorganic residues affect the magnitude of the signal. Hudnik *et al.*[4] stated that the presence of sulphuric acid added to mineral water samples is responsible for non-specific absorbance. Cruz and van Loon[5] studied the effect of some inorganic salts on the cadmium peak in the analysis of rock materials. They stated that the nature of the matrix effect may be physical (*e.g.*, occlusion, adsorption) or chemical (*e.g.*, compound formation), or it may be classified as spectral interference, which involves unresolved specific or non-specific absorbance.

Sturgeon *et al.*[6] evaluated atomisation energies in pure cadmium salt solutions and, by comparing them with relevant dissociation energies of corresponding compounds, suggested atomisation mechanisms. In the course of pyrolysis cadmium nitrate decomposes into cadmium oxide, which in the atomisation step dissociates into free atoms. Cadmium chloride, on the other hand, dissociates directly in the atomisation process.

The preparation of most biological and environmental samples for analysis involves the addition of inorganic acids, which are partly decomposed and volatilised and partly converted into metal salts, mainly those of sodium, calcium and magnesium. Their proportions vary considerably, depending on the sample material (Table 1), but may give some indications about the excesses that should be investigated. In this work the influence of excesses of inorganic acids and salts on the cadmium signal was studied, with particular emphasis on the mechanism of interferences.

Table 1. Content of cadmium and some major components in various matrices[7-11]

Element	Soils, p.p.m.	Sea water, p.p.m.	Blood serum, p.p.m.
Cd	0.1–1	0.01–0.5	0.002–0.01
Na	200–8000	10000	2500–3700
Ca	10–6000	400	60–120
Mg	50–2500	1300	20

* Presented at Euroanalysis V, 26–31 August 1984, Krakow, Poland.

Experimental

Instrument

A Perkin-Elmer Model 300A atomic-absorption spectrometer equipped with an HGA-72 graphite cuvette and a Model 159 recorder (Hitachi - Perkin-Elmer) was used. A cadmium hollow-cathode lamp (Perkin-Elmer) was used as the radiation source.

The volume of the injected sample was always 20 µl. The absorbance was measured at a wavelength of 228.8 nm using a band pass of 0.7 nm with the following thermal programme: drying at 370 K, 25 s; ashing at 620 K, 20 s; atomisation at 2070 K, 5 s; maximum power, 5 s. The atomisation curves were recorded at a heating rate of 25 K s^{-1} from ambient to the maximum temperature. The flow-rate of argon as a sheath gas was 1.5 l min^{-1}.

Reagents and Materials

Cadmium standard solutions

The stock standard cadmium solution (1 mg ml^{-1}) was prepared from pure cadmium nitrate. Working standard solutions (1 µg ml^{-1}) were prepared by appropriate dilutions of the stock standard solution.

Working standard cadmium solutions containing inorganic salts at concentrations of 0.5 mg ml^{-1} were prepared by diluting the stock solution with solutions of sodium sulphate, magnesium sulphate, sodium chloride, calcium chloride, magnesium chloride, sodium nitrate, calcium nitrate or magnesium nitrate. All the salts used were of analytical-reagent grade.

Acids

Dilute sulphuric, nitric and hydrochloric acids (1 and 0.1 M) were obtained by appropriate dilution of concentrated acids of high purity.

Procedure

The atomisation energy of cadmium was evaluated from the initial parts of the recorded atomisation curves for 20 ng of cadmium at a rate of temperature increase of 32 K s^{-1} in the range from 620 to 2070 K. The relationship between the logarithm of the absorbance, A, and the reciprocal of the absolute temperature, T, was evaluated using the least-squares method, with regression coefficients from 0.920 to 0.999. The atomisation energy was calculated as $E_a = fR\ln 10$, where f is the slope of the relationship $\log A$ *vs.* T^{-1} and R is the gas constant. The error of the determination of the

atomisation energy was evaluated as 10% on the basis of several subsequent measurements of the energies of known processes. The temperature measurements in the graphite tube carried out with a calibrated thermocouple were accurate to within 20 K.

Results and Discussion

Atomisation of cadmium in the graphite furnace depends significantly on the matrix. With solution samples this reflects the solution composition. The qualitative and quantitative character of absorbance peaks indicates that the atomisation processes are the same for cadmium sulphate and nitrate, the calculated atomisation energies being 264 and 272 kJ mol^{-1}, respectively, which are in good agreement with the value of 272 kJ mol^{-1} calculated by Sturgeon *et al.*[6] for cadmium nitrate. The coincidence with the value of the enthalpy of thermal dissociation of cadmium oxide[12] (281 kJ mol^{-1}) suggests that the rate-determining step is the decomposition of cadmium oxide:

$$CdO_{(g)} \rightarrow Cd_{(g)} + O_{(g)}$$

Cadmium oxide is formed as the product of thermal decomposition of the nitrate at 617 K. Such a mechanism seems probable for both cadmium sulphate and nitrate. Additional confirmation of such a process was given by Yasuda and Kakiyama,[13] who found the presence of sulphur dioxide in the graphite furnace at 673–773 K as a product of sulphate decomposition.

Atomisation of cadmium chloride takes place through thermal dissociation of gaseous CdCl molecules. The experimentally evaluated atomisation energy is 212 kJ mol^{-1}, which is in good agreement with literature data[12] for the reaction

$$CdCl_{(g)} \rightarrow Cd_{(g)} + Cl_{(g)} \ (\Delta H = 209 \text{ kJ mol}^{-1})$$

This agreement was obtained when the amount of cadmium in the sample was 20 ng. When 2 ng of cadmium chloride were atomised in a new graphite furnace, the free cadmium atoms appeared at 1080 K, with a maximum at 1400 K, which is analogous to the results for cadmium nitrate.

When cadmium chloride is repeatedly atomised from the same graphite tube the absorbance peak becomes distorted and a new maximum appears at 1560 K (Fig. 1). This effect is more pronounced when additional amounts of chlorine are introduced as hydrochloric acid (Fig. 2). A well shaped peak appeared in the presence of 50 µl of 1 M acid, with a maximum at 1560 K. The height of this peak increases as the amount of chlorine introduced is increased. The shape of the initial part of the absorbance peak and the calculated atomisation energy indicate that this peak corresponds to dissociation of CdCl in the gaseous phase.

These observations suggest that in a new graphite tube atomisation of $CdCl_2$ proceeds through cadmium oxide, but as chlorine accumulates in graphite and is subsequently released[14] the gas-phase dissociation of CdCl is promoted. To remove this memory effect of the graphite tube it is necessary to add 100 µg of 1 M nitric acid and heat the tube at 600 K for 40 s before injection of the cadmium sample solution (Fig. 3).

When the cadmium standard solution is atomised in the presence of inorganic oxy acids the temperature of peak appearance and the peak height may vary considerably, and these effects depend to some extent on the acid used. In all instances the peak maximum is shifted to higher temperatures but there appears to be no significant change in the mechanism of the process (Fig. 4). Such behaviour is explained by the shift of the equilibrium of thermal dissociation of cadmium oxide under the influence of oxygen formed in the decomposition of oxy acids. This shift increases for larger amounts of acid, but for concentrations higher than 1 M the peak height decreases and the precision deteriorates (Table 2). The effect of

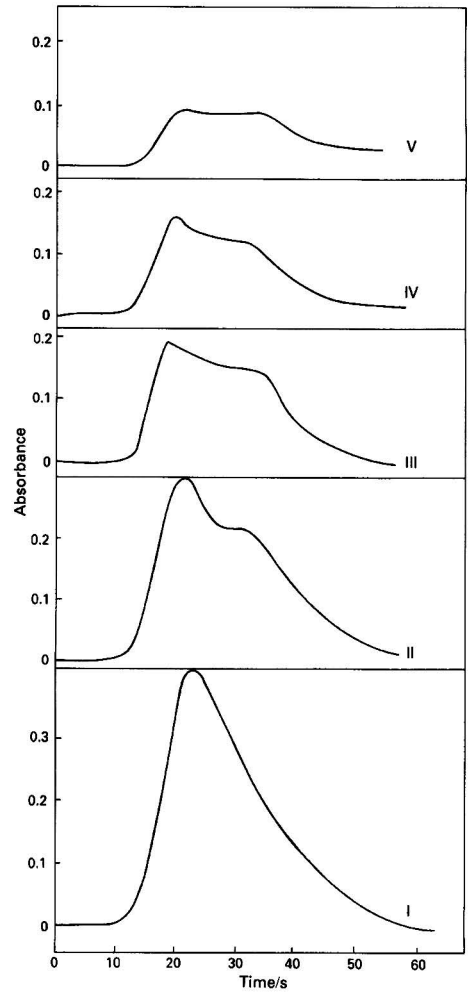

Fig. 1. Atomisation curves of 2 ng of cadmium as cadmium chloride in subsequent (I–V) measuring cycles

hydrochloric acid (Fig. 4), which, as mentioned above, promotes the CdCl dissociation mechanism, also eventually results in poorer precision and lower peak heights.

More complicated are the effects of a matrix composed of sodium, magnesium and calcium salts of the investigated acids. These effects were studied when the sample contained 0.5 mg ml^{-1} of the respective salt with the purpose of establishing the atomisation mechanisms in the presence of interfering substances. In most instances these salts decrease the absorbance peak, resulting in analytical errors (Table 3) and also in poorer precision.

When the matrix is composed of sodium, calcium or magnesium chloride both the peak height and the peak area decrease (Fig. 5) because of cadmium losses in the form of volatile chloride before the atomisation step. The evaluated atomisation energies (Table 4) support the suggestion that the dissociation of chloride is responsible for cadmium atom formation.

When the matrix is composed of calcium or magnesium nitrate (Fig. 6), both the peak height and the peak area decrease. In the presence of sodium nitrate the peak is split.

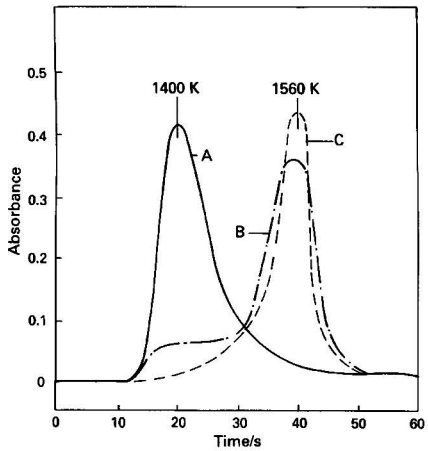

Fig. 2. Effect of various amounts of 1 M hydrochloric acid on the atomisation of 2 ng of cadmium. A, Cd standard (1400 K); B, Cd + 10 µl of HCl (1560 K); and C, Cd + 50 of µl HCl (1560 K)

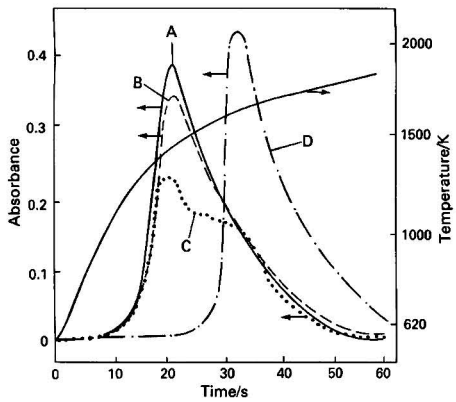

Fig. 3. Atomisation curves of 2 ng of cadmium. A, Cd standard; B, $CdCl_2$, first measuring cycle; C, $CdCl_2$, third measuring cycle; and D, $CdCl_2$, after treatment of the tube with 100 µl of 1 M HNO_3 and heating at 600 K for 40 s

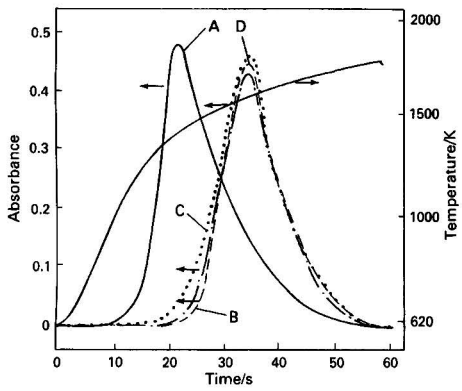

Fig. 4. Effect of 0.1 M inorganic acids on the atomisation of 2 ng of cadmium. A, Cd standard; B, Cd + HNO_3; C, Cd + HCl; and D, Cd + H_2SO_4

Table 2. Effect of inorganic acids on the peak height for 0.5 ng of cadmium. Mean values of six measuring cycles

Sample	Peak height/mm	Relative absorbance	Relative standard deviation, %
Cd (standard)	60	1.00	1.1
Cd + 1 M H_2SO_4	56	0.93	1.5
Cd + 1 M HNO_3	47	0.78	1.1
Cd + 1 M HCl	34	0.57	1.7

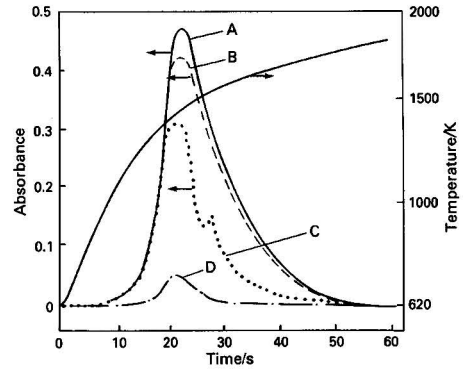

Fig. 5. Effect of 0.5 mg ml^{-1} chloride salts on the atomisation curves of 2 ng of cadmium. A, Cd standard; B, Cd + NaCl; C, Cd + $CaCl_2$; and D, Cd + $MgCl_2$

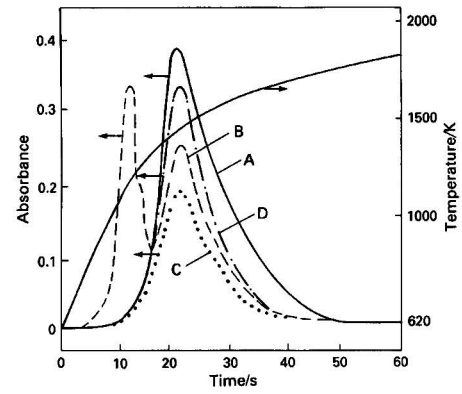

Fig. 6. Effect of 0.5 mg ml^{-1} nitrate salts on the atomisation curves of 2 ng of cadmium. A, Cd standard; B, Cd + $NaNO_3$; C, Cd + $Ca(NO_3)_2$; and D, Cd + $Mg(NO_3)_2$

The new peak at 1200 K is poorly reproducible and is independent of the cadmium concentration in the sample. It may be due to non-specific absorption. The normal peak at 1400 K depends on the cadmium concentration. The evaluated atomisation energy in the presence of sodium or magnesium nitrate differs by not more than 10% from the enthalpy of thermal dissociation of cadmium oxide, which confirms the following mechanism of atomisation:

$$CdO_{(s)} \rightarrow Cd_{(s)} + O_{(g)}$$
$$Cd_{(s)} \rightarrow Cd_{(l)} \rightarrow Cd_{(g)}$$

In the presence of calcium nitrate the atomisation energy is significantly higher than expected for this process. This may be attributed to the simultaneous evaporation of calcium and cadmium nitrates.

Table 3. Effect of inorganic salts (cation concentration 0.5 mg ml^{-1}) on the peak height for 0.5 ng of cadmium. Mean values of six measuring cycles

Parameter	Cd (standard)	NaCl	$CaCl_2$	$MgCl_2$	$NaNO_3$	$Ca(NO_3)_2$	Na_2SO_4	$MgSO_4$
Peak height/mm ..	60	57	34	23	61	47	63	51
Relative absorbance ..	1.00	0.95	0.57	0.38	1.05	0.78	1.05	0.85
Relative standard deviation, %	1.1	3.0	4.0	1.4	1.5	1.8	2.2	1.5

Table 4. Evaluated atomisation energies, E_a, in comparison with energies of probable processes, E_L.[12] The experimental values were evaluated for 20-ng cadmium samples containing 0.5 mg ml^{-1} of inorganic salt. The atomisation curves were recorded at a rate of temperature increase of 32 K s^{-1}

	Sodium			Calcium			Magnesium		
Sample	E_a/ kJ mol^{-1}	E_L/ kJ mol^{-1}	$\frac{E_L - E_a}{E_L} \times 100$, %	E_a/ kJ mol^{-1}	E_L/ kJ mol^{-1}	$\frac{E_L - E_a}{E_L} \times 100$, %	E_a/ kJ mol^{-1}	E_L/ kJ mol^{-1}	$\frac{E_L - E_a}{E_L} \times 100$, %
Nitrate ..	420	381	−10	508	381	−33	403	381	−6
Chloride ..	240	209	−15	220	209	−5	230	209	−10
Sulphate ..	291	281	−4	—	—	—	167	202	+17

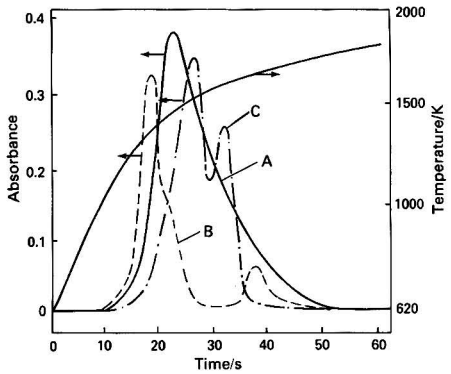

Fig. 7. Effect of 0.5 mg ml^{-1} sulphate salts on the atomisation curves of 2 ng of cadmium. A, Cd standard; B, Cd + Na_2SO_4; and C, Cd + $MgSO_4$

Addition of magnesium or sodium sulphate complicates the atomisation process by splitting the absorbance peak of cadmium. The second peak in these instances follows after the normal cadmium peak and, in the presence of sodium sulphate, occurs at 1800 K (Fig. 7). Only the first peak, which appears at a lower temperature (1300 K), depends on the cadmium concentration. The atomisation energy (Table 4) indicates that this peak may be connected with dissociation of cadmium oxide in the gas phase, whereas the second peak results from the non-specific absorption, as has been confirmed by measurements of a non-absorbed wavelength at 232 nm.

Both peaks formed in the presence of magnesium sulphate depend on cadmium concentration and no non-specific absorption was observed in this instance. This suggests that cadmium atomises in two stages. The evaluated atomisation energy for the first peak at 1450 K is 167 kJ mol^{-1}, which is evidently lower than the energy determined for other processes. [The experimentally found energy values have a tendency to be higher than the literature values for corresponding processes (Table 4).] It is probable that in the presence of an excess of magnesium sulphate, cadmium sulphate undergoes reduction to sulphide, which in turn dissociates into cadmium atoms:

$$CdS_{(g)} \rightarrow Cd_{(g)} + S_{(g)} \ (\Delta H = 202 \text{ kJ mol}^{-1})$$

The relatively large and positive error between the experimentally determined atomisation energy and the bond dissociation energy given in the literature throws doubt on the proposed mechanism, but our present knowledge does not allow for a better hypothesis. The shape of the split atomisation curve does not allow evaluation of the atomisation energy for the second peak, as the peak maximum does not correspond to any of the known processes of cadmium atomisation. It cannot be excluded that retarded decomposition of cadmium oxide occurs at this stage.

Conclusions

The investigation of matrix effects on the determination of cadmium indicates that the interferences are connected mainly with the presence of anions. Their presence results in the formation of various compounds during the thermal decomposition stage. Depending on the initial sample composition, the cadmium atoms may be formed through dissociation of cadmium oxide, chloride or possibly sulphide. The anionic effect is modified by the influence of cations, which primarily interfere physically by changing the volatility of individual components or by occlusion of cadmium in the less volatile matrix.

A knowledge of matrix effects may help the analyst to select mineralisation conditions that lead to optimum responses in the determination of cadmium using electrothermal atomisation. The presence of commonly used sulphuric acid causes significant errors, especially when the samples contain a large proportion of magnesium. It seems to be advantageous to convert samples into the nitrate form and to measure peak area instead of peak height.

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Evaluation of a Slurry Technique for the Determination of Lead in Spinach by Electrothermal Atomic-absorption Spectrometry

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A simple method for the determination of lead in spinach involving minimum sample preparation is described. The powdered spinach is suspended in a thixotropic thickening agent, Viscalex HV30, and the slurry is injected directly into the electrothermal atomiser. Oxygen is introduced during the ashing stage to allow the use of higher ash temperatures and to avoid the build up of carbonaceous residue in the tube. Concentrations of powdered spinach up to 10% *m/V* can be tolerated in the suspension. Good agreement was achieved between results obtained by a standard additions procedure and by direct calibration with aqueous standards, and also by an alternative wet digestion procedure.

Keywords: *Lead; spinach; atomic-absorption spectrometry; slurry; electrothermal atomisation*

Atomic-absorption spectrometry with electrothermal atomisation has become one of the most popular techniques for the determination of trace elements in foodstuffs. One disadvantage of the technique, however, is that the sample is almost exclusively required to be in solution, and this usually involves time-consuming and often complex dissolution procedures prior to the analysis. Several wet oxidation[1-3] and dry ashing[4-6] methods have been proposed for the decomposition of foodstuffs, some involving a final pre-concentration step using chelation - solvent extraction.[7] Although many of these procedures are reliable and reproducible, a large proportion of the analysis time is spent at the sample preparation stage.

The direct determination of trace metals using solid sampling, slurries or suspensions offers potential advantages in analytical speed and convenience compared with dry ashing or wet digestion. Solid sampling obviously removes the need for time-consuming dissolution procedures and has the attraction that the analysis can be carried out without the addition of reagents. This limits the risk of contamination and avoids dilution of the sample. Unfortunately, direct solid sampling has several limitations and so only relatively few procedures have been reported[8-13] for the determination of lead and other trace elements in foodstuffs. With direct solid sampling, molecular absorption and/or scattering from the matrix may produce a high background signal, which is often too large for the commonly used deuterium arc background correction system to compensate adequately. Also, if the vaporisation characteristics of the analyte and matrix are too similar for complete separation, effective elimination of the matrix in the ashing step is not possible without partial or total loss of the analyte, particularly with volatile metals such as lead. Further difficulties arise from sample homogeneity, calibration of the instrument and the need for repeated micro-weighings for a single analysis.

The use of slurries and suspensions combines the advantages of solid and liquid sampling, because the sample can be transported into the atomiser by conventional procedures following minimum pre-treatment of the sample. Slurry/suspension methods have been used successfully by several workers for the determination of a variety of elements in animal tissue,[14] pigments[15] and geological materials.[16-18] Many of these studies have employed flame atomic-absorption spectrometry, but standardisation is difficult with this technique, as atomisation efficiency and hence sensitivity are acutely dependent on the particle size of the sample powder.[19] Fuller *et al.*[18] reported that larger particle sizes can be tolerated in electrothermal atomisation and they described a technique for the direct analysis of powdered materials in which a thixotropic thickening agent was employed to stabilise the suspensions. The thickening agent used was Viscalex HV30, an acrylic copolymer containing carboxyl groups, which is supplied in the form of an acidic low-viscosity emulsion. On dilution and neutralisation the emulsion produces a viscous gel over the pH range 6–10.

The slurry/suspension technique appears to be potentially applicable to the analysis of foodstuffs as many foods can be successfully freeze-dried and ground to a fine powder. The technique described by Fuller *et al.*[18] has been investigated for the determination of lead in spinach and the results are reported in this paper. A general problem encountered when introducing biological materials directly into the furnace is the production of a carbonaceous residue in the tube. The residue may not be visible after a few determinations but will eventually affect the sensitivity of the analysis and may partially block the optical beam, thus increasing the non-specific absorption signal, which in turn degrades the analytical precision. It has been shown[10,20-22] that the introduction of oxygen during the ashing step aids in the oxidation of an organic matrix by converting the charring step into an oxidative decomposition process. This eliminates the build-up of carbonaceous residue produced when charring organic materials in an inert gas atmosphere. Additionally, the incorporation of an oxygen ashing stage allows the use of higher ashing temperatures for lead without loss of the analyte. The mechanism involved in this process has been discussed previously by Salmon and Holcombe.[22]

This paper describes a simple, rapid method for the determination of lead in spinach, which combines a slurry procedure to minimise sample preparation with an oxygen ashing step that helps remove much of the organic matrix before the atomisation step.

Experimental

Apparatus

Lead determinations were performed with a Perkin-Elmer Model 3030 atomic-absorption spectrometer equipped with an HGA-500 graphite furnace, an AS-40 autosampler and an Anadex DP-9001B printer. Deuterium arc background correction was used throughout. All absorbance measurements were made at the 283.3 nm Pb wavelength using a lead hollow-cathode lamp (Juniper) and a band width of 0.7 nm. Pyrolytically coated graphite tubes were used for all of the determinations and measurements were based on peak height, using a read time of 1.5 s.

The ground spinach was milled in a Fritsch Pulverisette and homogenised with water in a Jencons Uni-Form homogeniser/grinder.

Reagents

Viscalex HV30. Allied Colloids Ltd., Bradford.

NOPCO NPZ antifoaming agent. Diamond Shamroch Process Chemicals Ltd., Leeds.

Ammonia solution, 35% (*sp. gr.* 0.88). AnalaR grade.

Lead stock solution, 1000 μg ml^{-1}. Spectrosol grade, BDH Chemicals Ltd., Poole.

Samples

The spinach sample used throughout was collected and prepared at the MAFF Food Laboratories, Norwich, where it was freeze-dried and ground in a Moulinex coffee grinder to give a coarse powder.

Procedure for the Preparation of a Spinach Slurry

The freeze-dried, powdered spinach was ground in an agate ball-mill for 1.5 h to reduce any small stalks and larger particles present to a uniform powder. A mass of spinach was weighed (0.5–10 g), transferred into the tissue grinder and homogenised with 10–20 cm^3 of distilled water. The slurry was transferred into a 100-cm^3 calibrated flask where 2–3 cm^3 of Viscalex HV30 were added, and the mixture was neutralised with ammonia solution. The flask was continuously agitated during the formation of the slurry and then the thickened sample was diluted to 100 cm^3 with distilled water. If problems with foaming were found to occur, a few drops of antifoaming agent were added before the slurry was diluted to 100 cm^3.

Standard addition calibration graphs were prepared by transferring 10-cm^3 aliquots into four acid-cleaned glass test-tubes. The appropriate amount of a 2 μg ml^{-1} lead stock solution was added to each test-tube with a 50–200-μl calibrated, adjustable micropipette. Small volumes of the viscous mixtures were then transferred into autosampler cups.

The atomiser conditions were optimised for the analysis of the slurry solution and were not significantly different from those found for aqueous solutions. The parameters used for all determinations are given in Table 1.

Results and Discussion

Stability of the Slurry

Preliminary experiments revealed that the stability of a slurry depended on the particle size of the powder, the concentration of spinach in the slurry and the concentration of Viscalex used to form the suspension. Initially an agate mortar and pestle was used to grind the powdered spinach prior to slurry formation. However, the suspensions were only stable for short periods, especially at higher spinach concentrations. To reduce the particle size, samples were ground in an agate ball-mill for 1.5 h, which allowed the preparation of spinach slurries in the 1–10% *m/V* range that were stable for several hours. The particle size distributions of the spinach powder ground by the mortar and pestle, and by the ball-mill, were measured using a Coulter Counter (Coulter Electronics Ltd.) fitted with a 100- or 200-μm orifice. The cumulative distribution curves obtained for both powders are shown in Fig. 1. With the mortar and pestle only 80% of the spinach particles were less than 50 μm in diameter, and particles as large as 100 μm were measured for this sample. After grinding in a ball-mill for 1.5 h, all the particles were 50 μm in diameter or less, with 75% of these below 17 μm. It is important to note that the spinach particles swell in the electrolyte prior to measurement. The particle size of the prepared spinach powders before slurry formation was therefore significantly lower than indicated by the results in Fig. 1.

It was found that a 1% *V/V* concentration of Viscalex as used by Fuller and Thompson[17] was insufficient to produce a stable suspension of spinach powder. For slurry concentrations in the range 0.5–2% *m/V* a 2% *V/V* Viscalex solution was employed, but at higher spinach concentrations it was necessary to increase the volume of Viscalex added to the solution. For example, a stable suspension of 10% *m/V* spinach required a 3% *V/V* Viscalex medium.

Initially, difficulty was encountered in dispensing viscous slurry volumes with the AS-40 autosampler. The Viscalex solution droplets would frequently remain attached to the autosampler capillary and drop on to the inside wall of the tube near the injection hole, with consequential impairment of analytical precision. To overcome this problem, the capillary delivery position was carefully adjusted and the tubing frequently cleaned with acetone.

Optimisation of Furnace Conditions

Fig. 2(*a*) compares the effect of the variation of ashing temperature on the lead atomic-absorption signal for a 30 μg l^{-1} aqueous lead solution with that for lead in a slurry containing 1% *m/V* of spinach plus 40 μg l^{-1} of Pb. In both instances the introduction of oxygen allows a significant increase in the ashing temperature without analyte loss, but the extension of the ashing temperature range is less with the spinach slurry. Nevertheless, a temperature of 800 °C for 30 s was selected as the optimum ashing temperature for lead in a 1% *m/V* spinach slurry. The ashing time was increased to 90 s for a 10% *m/V* spinach dispersion to remove the increased level of matrix components.

The effect of the variation of atomisation temperature on the lead signal from a spinach slurry using the optimum ashing temperature of 800 °C is shown in Fig. 2(*b*). The optimisation

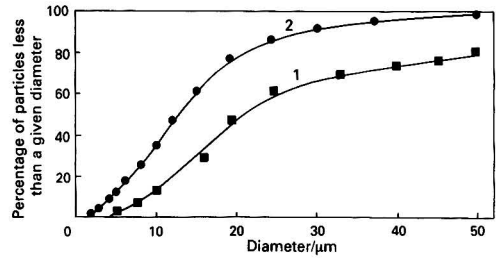

Fig. 1. Cumulative distribution plot for the particle size of spinach powder ground in 1, an agate mortar and pestle and 2, a ball-mill for 1.5 h

Table 1. HGA-500 programme for the determination of lead using slurry atomisation. Injection volume, 20 μl

Stage	Temperature/°C	Ramp time/s	Hold time/s	
Dry	120	1	40	
	130	1	20	
Ashing*	800	1	30	(20 cm^3 min^{-1} O_2)
Purge	300	1	20	(300 cm^3 min^{-1} N_2)
Atomise	1800	0	3	(0 cm^3 min^{-1} N_2)
Clean	2400	1	2	

* Conditions for up to a 6% *m/V* spinach slurry; hold time increased to 90 s for a 10% *m/V* slurry.

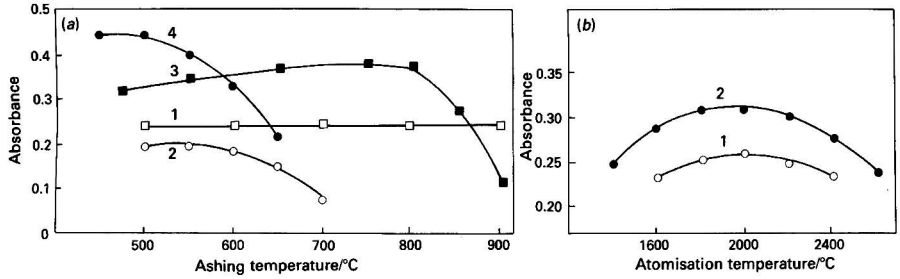

Fig. 2.(*a*) Effect of the ashing temperature on the peak-height absorbance for 1 and 2, a 30 μg l^{-1} aqueous Pb solution, and 3 and 4, Pb in a slurry containing 1% *m/V* of spinach plus 40 μg l^{-1} of Pb using oxygen ashing (1 and 3) and nitrogen ashing (2 and 4); 20-μl injections. (*b*) Effect of the atomisation temperature on the peak-height absorbance for 1, 30 μg l^{-1} of aqueous Pb solution and 2, Pb in a 1% *m/V* spinach slurry plus 30 μg l^{-1} of Pb; oxygen ashing; 20-μl injections

Table 2. Lead and background absorption signals obtained for spinach concentrations in the range 1–10% *m/V*

Concentration of spinach powder in the slurry, % *m/V*	Lead signal		Background signal		
	Peak height/A	Peak area/A s	Peak height/A	Peak area/A s	At lead peak height/A
1	0.055	0.020	0.145	0.087	0.022
3	0.181	0.049	0.500	0.247	0.027
6	0.338	0.083	0.786	0.381	0.027
10	0.462	0.095	1.006	0.504	0.027

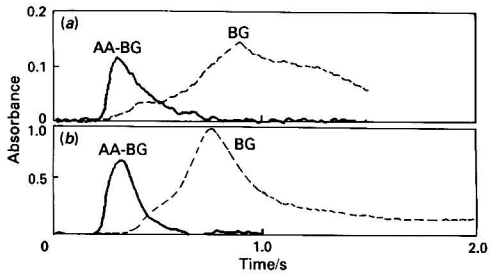

Fig. 3. Absorbance *versus* time profiles for Pb in a slurry containing (*a*) 1% *m/V* of spinach powder and (*b*) 10% *m/V* of spinach powder; 20-μl injections

curve obtained was similar to that for aqueous lead solutions atomised under identical conditions. At the seven temperatures investigated there was no pronounced difference in the shape and precision of the slurry lead peaks. Optimum atomisation conditions of 1800 °C, at maximum power heating with a hold time of 3 s, were therefore selected on the basis that this temperature gave the maximum peak-height signal.

Investigation of Matrix Interferences

Influence of background absorption on lead measurements

The background correction system on the Perkin-Elmer 3030 measures the maximum peak-height atomic-absorption signal and subtracts the background signal at that point in time. It is evident from Fig. 3 that the background peak occurs relatively late in the atomisation cycle and that the background signal can be effectively time resolved from the maximum peak height of the analyte. However, as the peak-area signal cannot be time resolved in the same way, the total background signal within the integration limits of 0–1.5 s has to be corrected for. Table 2 lists the peak-height and peak-area signals for lead and the relevant background measurements obtained for different concentrations of spinach powder in the slurry. It is clear that although the background signal increases with increasing concentration of spinach powder in the slurry, the time differentiation between the analyte and background signals (see Fig. 3) ensures that the background signal at the maximum of the analyte signal remains very small even at a spinach content of 10% *m/V* in the slurry. The background correction system compensates adequately for the small background signal at the lead peak maximum, and so accurate net peak-height measurements are obtained. At spinach powder concentrations above 3% *m/V*, the maximum background absorption exceeds the normal background correction capability of the instrument, which results in erroneous analyte peak-area measurements. This is illustrated by the results in Table 2. For slurry concentrations up to 10% *m/V*, the lead peak height increases almost linearly in relation to the level of spinach in the suspension. However, no such relationship exists for the corresponding peak-area signals. It appears that overcompensation for the matrix background absorption has occurred on atomisation of the 6 and 10% *m/V* slurry samples. Although shorter integration times of the order of 0.75–0.85 s might be used to remove the effect of the large background signal on the peak-area measurement, there is sufficient overlap with the tail of the lead peak to suggest that this may cause significant error. Overall, these results suggest that peak-height measurements must be used for lead determination, particularly at high slurry concentrations.

Investigation of chemical interferences

Standard additions graphs were prepared using slurries containing different concentrations of spinach powder, to observe any enhancement or suppression of the analyte signal compared with aqueous lead standards. Illustrative graphs are presented in Fig. 4. It was found that the aqueous calibration and standard additions graphs were almost but not exactly parallel. The slopes obtained differed on average by about 5%, which is similar to the analytical precision of the method.

Determination of lead in spinach

The concentration of lead in the spinach sample used throughout this work was calculated from each of the standard additions graphs given in Fig. 4 and also by comparison with

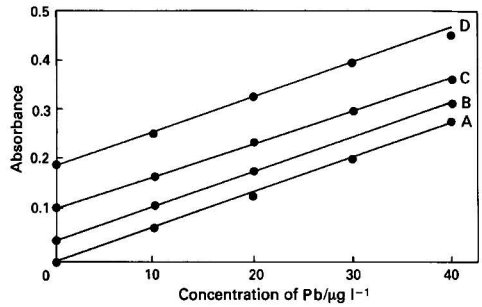

Fig. 4. A, aqueous Pb calibration graph and B–D standard additions graphs for Pb in slurries containing (B) 0.5, (C) 1.0 and (D) 2.0% *m/V* of spinach powder

the aqueous standard calibration graph. The results are presented in Table 3. A similar lead concentration was derived for each of the slurry suspensions, and by both the standard additions and direct aqueous calibration procedures. The mean lead content of the spinach powder obtained by the slurry method was found to be in reasonably close agreement with the concentration determined by atomic-absorption spectrometry with electrothermal atomisation at the MAFF Food Analysis Laboratories, Norwich, following wet digestion of the sample. For spinach slurries up to at least the 2% *m/V* level, it appears that with oxygen ashing, direct analysis against a calibration graph prepared from aqueous standards is just as accurate as analysis by standard additions.

In order to obtain the lowest possible detection limits using the slurry technique, it is necessary to increase the concentration of spinach powder in the slurry to greater than 2% *m/V*. It is possible, however, that the gain in detection limit may be impaired if the precision of the lead atomic-absorption signals is adversely affected by the increased level of sample matrix. To investigate the influence of the spinach concentration on precision, suspensions containing 1, 3, 6 and 10% *m/V* of spinach were prepared in the appropriate concentration of Viscalex solution that gave a stable slurry. The mean absorbance and the relative standard deviation values were calculated from five measurements at each concentration. The results are given in Table 4. During initial experiments, the precision at the 10% *m/V* level was poor owing to the adoption of a 30-s ashing time in the HGA-500 atomiser programme, which was insufficient to remove the additional matrix. This resulted in the building-up of a carbonaceous residue, which affected both the sensitivity and precision of analysis. When the ashing time was increased to 90 s the removal of the spinach matrix was significantly improved and the relative standard deviation decreased from 15 to 2%. In general, the precision values obtained for the spinach slurry signals given in Table 4 were approximately a factor of two greater than those of aqueous lead solution measurements of equivalent magnitude (*e.g.*, relative standard deviation of 4.0% for 0.05 A, 1.1% for 0.4 A).

Approximate detection limits were calculated from the mean absorbance and relative standard deviation values obtained for each spinach slurry concentration and the results are presented in Table 4. Although the values cannot be directly compared as they have been calculated at different absorbance values, and do not represent true detection limits as the concentrations used are too high owing to the level of lead in the spinach sample used, they clearly indicate that an increase in spinach concentration allows lower detection limits to be achieved.

To check the over-all precision of the procedure, five 3% *m/V* spinach slurries were individually prepared and analysed. The relative standard deviation was calculated from the means of the five absorbance values obtained from each spinach sample. The precision of the method was 3%.

Table 3. Results obtained for the determination of lead in a spinach sample using slurry atomisation

Concentration of spinach powder in slurry, % *m/V*	Lead content of spinach powder/μg g⁻¹	
	Standard additions	Aqueous calibration
0.5	1.30	1.46
1.0	1.59	1.59
2.0	1.48	1.43
Mean*:	1.46 ± 0.15	1.49 ± 0.09

* Mean value obtained using wet digestion procedure = 1.36 ± 0.08 μg g⁻¹.

Table 4. Detection limits for lead with increasing concentrations of spinach powder in the slurry

Concentration of spinach powder in slurry, % *m/V*	Mean absorbance	Relative standard deviation, %	Calculated detection limit*/ μg g⁻¹
1	0.055	9.8	0.36
3	0.175	2.2	0.104
6	0.338	4.5	0.105
10	0.462	2.1	0.047

* Calculated on the basis of 2σ using the data in columns 2 and 3 (see text for explanation).

Conclusions

Although direct analysis of solid samples of foodstuffs might offer advantages in terms of sensitivity, the additional time required for the weighing and mechanical transfer of small amounts of the sample is a major disadvantage, particularly if duplicate analyses are required for more than one element. The slurry technique is an attractive compromise, offering simplicity of sample preparation with the convenience of a permanent or semi-permanent sample "solution" for repetitive analysis. The results in this paper indicate that the use of higher powder concentrations is a practical possibility, at least as far as lead is concerned, and that lower detection limits are then feasible. Further research on the slurry procedure will be concentrated on extending the method to other foodstuffs and the analysis of materials with lower lead contents.

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Simultaneous Multi-element Analysis by Continuum Source Atomic-absorption Spectrometry with Graphite Probe Electrothermal Atomisation*

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An automatic graphite probe atomiser has been used in conjunction with a continuum source atomic-absorption spectrometer system (SIMAAC) for the simultaneous determination of up to 15 elements. The influence of the atomisation stage temperature on the magnitude and precision of peak-height and peak-area signals has been investigated for a range of elements. A compromise atomisation temperature of 2700 °C was found to give efficient atomisation and good peak-height sensitivity for all the elements studied. In comparison with tube-wall and platform atomisation, probe - SIMAAC detection limits (0.01–7.0 μg l^{-1}) are equivalent or superior, except for the less volatile elements (*e.g.*, V, Mo). Analysis of NBS Standard Reference Materials (bovine liver, rice flour and wheat flour) indicated that the probe procedure was more accurate than tube-wall atomisation methods, but less precise than platform atomisation methods.

Keywords: *Continuum source atomic-absorption spectrometry; probe atomisation; simultaneous analysis; compromise conditions*

To achieve simultaneous multi-element analysis by atomic-absorption spectrometry (AAS), it is necessary to use a high-intensity continuum source in combination with a high-resolution polychromator. The development of such a system, known as SIMAAC, has been described in a number of recent publications and the analytical characteristics of this AAS technique are now well established.[1–4] Although compatible with both flame and electrothermal atomisation, the advantages provided by the SIMAAC technique are most apparent in the simultaneous measurement of elements in the transient sample vapour produced by a graphite furnace atomiser. With electrothermal atomisation, simultaneous multi-element detection limits achieved by SIMAAC are comparable to those obtained by conventional single element ETA-AAS, when the atomic-absorption wavelength is greater than 280 nm.[5–7] In addition, automatic background correction, an essential requirement in most ETA analyses, is an intrinsic feature of the SIMAAC instrument and is equivalent in performance to correction procedures based on the Zeeman effect[8] and the Smith - Hieftje method.[9]

In conventional ETA-AAS, different furnace programmes are generally required for the determination of several elements in a sample. With SIMAAC, it is clearly necessary to select a single atomisation temperature that will give reasonable sensitivity for elements of widely differing volatility. A previous investigation[5] revealed that for both tube-wall and platform atomisation, a temperature of 2700 °C or greater was required for the simultaneous determination of Co, Cr, Cu, Fe, Mn, Mo, Ni, V and Zn in acidified water samples. However, at this temperature neither mode gives atomisation conditions that are totally satisfactory. With tube-wall atomisation, atoms of volatile elements are produced when the expanding furnace vapour is still at a comparatively low temperature. For elements such as Cd, Pb and Zn, vapour-phase chemical interference effects are therefore inevitable if the sample contains appreciable concentrations of halide salts. The degree of vapour-phase interference is much lower when platform atomisation is employed, as is illustrated by the greater accuracy of peak-area SIMAAC determinations obtained using stepped atomisation from a platform.[6] However, the rate of atomisation from a platform is reduced compared with the tube-wall mode, which results in degradation of both peak-height and peak-area detection limits.[5] It has also been shown that with a platform the magnitude of SIMAAC absorption signals is more susceptible to changes in atomisation temperature compared with wall atomisation.[5]

A variety of alternative atomiser concepts have been proposed to overcome the limitations of tube-wall and platform atomisation.[10–14] Most of the atomisers are designed to achieve conditions of isothermal atomisation, as originally recommended by L'vov,[15] through independent control of the procedures used to volatilise the sample and cause atomisation. Frech *et al.*[16] pursued this strategy and constructed a furnace that consisted of a graphite tube and sample cup that were heated independently by two power supplies. A similar approach has been investigated by Siemer.[17] An alternative procedure that can be applied with existing commercial atomisers involves the introduction of a sample probe into a preheated furnace tube. This is best achieved by use of thin strips of graphite on to which the sample droplet is deposited either inside[18] or outside[19] the atomiser tube.

Automatic graphite probe assemblies have been developed for use with Perkin-Elmer HGA 500[20] and Pye Unicam SP 9[21] electrothermal atomisers. Totally pyrolytic graphite or pyrolytically coated microporous glassy carbon probes were used with both systems. The sample droplet was deposited on the probe head and dried and ashed within the atomiser, and the probe was moved into and out of the tube via a small slot cut on the tube wall. The results obtained with both probe atomisers suggest that most elements are rapidly volatilised from the probe surface and experience the same high vapour

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Table 1. Furnace programme for probe - SIMAAC simultaneous multi-element analysis

Stage	Temperature/°C*	Time/s	Ramp
Dry	450	45	5
Ash	—	—	—
Pre-heat	2700 RC	5	0
Atomise†	2700 GS	5	0

* RC, recorder function selected, in this instance to initiate removal of the probe. GS, gas stop during atomisation.

† 10 cm³ min⁻¹ Ar gas flow; other stages 300 cm³ min⁻¹.

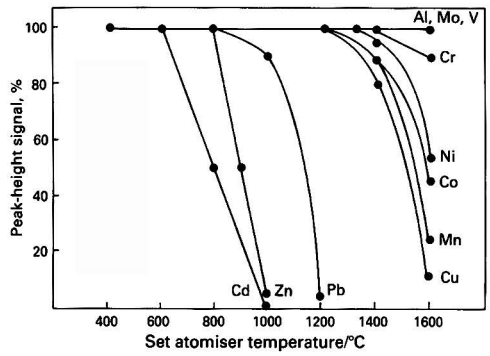

Fig. 1. Ash stage temperature optimisation graphs for selected volatile and involatile elements by probe atomisation; plot of relative peak-height signal against set atomiser tube temperature

temperature irrespective of the atom appearance temperature. In addition, sensitivities and detection limits obtained with probe atomisation are similar to, if not better than, those for conventional ETA procedures. Probe atomisation may therefore have considerable potential for use in simultaneous multi-element analysis by continuum source AAS.

This paper presents the results of an investigation to determine whether rapid atomisation from a probe into an isothermal environment allows easy selection of a uniform set of optimised furnace conditions for simultaneous multi-element analysis with the SIMAAC instrument. The advantages of the probe technique have been assessed in comparison with tube-wall and platform atomisation by analysis of National Bureau of Standards (NBS) Standard Reference Materials (bovine liver, rice flour and wheat flour).

Experimental

Instrumentation

The SIMAAC system has been described previously.[1-4] The instrument consists of a 300-W xenon arc lamp as the primary source, an échelle polychromator modified for wavelength modulation and a PDP 11/34 minicomputer. The computer generates the modulation waveform, stores intensity data and calculates the background-corrected absorbance at the 16 pre-selected analyte wavelengths. The current suite of wavelengths and slit dimensions incorporated in the spectrometer multi-element cassette has been reported recently.[5] In this work, absorbance measurements were obtained simultaneously for Al (396.2 nm), Ca (422.7 nm), Cd (228.8 nm), Co (240.7 nm), Cr (357.9 nm), Cu (324.8 nm), Fe (248.3 nm), K (404.4 nm), Mg (285.2 nm), Mn (279.5 nm), Mo (313.3 nm), Ni (232.0 nm), Pb (283.3 nm), V (318.4 nm) and Zn (213.9 nm).

Electrothermal atomiser

A Perkin-Elmer HGA 500 electrothermal atomiser was used throughout this work. The atomiser was modified for use with an automatic probe assembly.[20] The automatic probe consisted of a solenoid activated by a simple d.c. power supply. The action of the solenoid controlled the motion of the graphite, probe, which was introduced into the atomiser via a slot cut below the injection port of a pyrocoated tube. Automation of the system was accomplished by connecting the solenoid power supply via a relay to the furnace programmer. In its rest position the graphite probe head was situated inside the atomiser. An AS-1 autosampler was used to deposit the samples on the probe head. Sample droplets were dried and ashed with the probe head inside the furnace tube. The solenoid was then activated prior to the atomisation step and the probe removed from the furnace. Once constant temperature conditions had been established, the solenoid was deactivated automatically and the probe returned into the atomiser by means of a spring. After the normal probe sequence the probe remained inside the atomiser tube and the cycle was repeated for the next sample. The probes used in this study were T-shaped and varied in thickness from 200 to 300 μm. The probe head measured 4 × 4 mm and the stem was 35 mm long × 3 mm wide. Most measurements were obtained with probes made from totally pyrolytic graphite or pyrocoated microporous glassy carbon. Both probes have similar atomisation characteristics.[21]

Atomisation parameters

The programme used for the analysis of NBS Standard Reference Materials is given in Table 1. All temperatures are nominal values displayed by the HGA 500. Prior to selection of the compromise set of atomisation parameters, the optimum conditions for each individual element were investigated, as follows.

Dry stage. With probe atomisation the sample droplet is evaporated by convection of the hot furnace gases. The temperature and the time values were selected to ensure gentle removal of the solvent without excessive boiling. Previous SIMAAC studies have involved the use of sample and standard solutions that contained 5% nitric acid. In the present work it was not possible to use solutions of this acid concentration owing to the lower surface tension of the solution, which caused the droplet to spread along the probe stem during the dry stage. To overcome the problem, it was necessary to reduce the acid concentration to between 0.05 and 0.5% HNO_3 and to use 10-μl sample volumes.

Ash stage. Ashing temperature optimisation graphs obtained for the individual elements considered in this study are illustrated in Fig. 1. In simultaneous multi-element analysis, selection of the ashing temperature is dictated by the most volatile elements to be determined. Hence the inclusion of cadmium in the list of test elements effectively limited the ashing temperature to a maximum of 600 °C, too low for efficient removal of matrix from most types of samples. It was decided, therefore, to omit an ash stage for most of the signal measurements rather than remove cadmium and the other volatile elements (Pb, Zn) from the study. Omission of an ash stage represented the "worst case" situation for evaluation of the probe atomisation procedure in that none of the matrix was removed prior to the atomisation stage.

Atomisation stage. Prior to selection of a compromise atomisation temperature, it was necessary to investigate the influence of the tube temperature on the magnitude and precision of peak-height and peak-area signals for elements of different volatility. Individual optimisation studies were therefore conducted for each element as described below.

Calibration Standard Solutions

Multi-element calibration standards were prepared from multi-element stock solutions (Spex Industries). A series of solutions covering the concentration range 1 μg l^{-1} to 10 mg l^{-1} were prepared, each diluted to give a final nitric acid concentration of 0.05 or 0.5% *V/V*. Two standards per decade of concentration were prepared and each solution contained the same concentration of all elements.

Analysis of NBS Standard Reference Materials

Three NBS Standard Reference Materials were analysed: bovine liver (1577)[22], rice flour (1568)[23] and wheat flour (1567).[24] The required amount of reference material was weighed into a test-tube and wet ashed using an HNO_3 - H_2O_2 dissolution procedure.[25] The digests were then diluted to provide acceptable concentration levels for furnace analysis. Final dilutions for rice flour and wheat flour were 1 g to 1000 cm^3 and for bovine liver the dilution factor was 0.5 g to 1000 cm^3. Analyte concentrations in the diluted sample solutions were determined by reference to the signals obtained for multi-element standard solutions.

Detection Limits

Detection limits were computed as the concentration that gave a signal equivalent to three times the standard deviation of the base-line absorbance obtained on atomisation of the reagent blank.[5,6]

Results and Discussion

Selection of Compromise Atomisation Temperature

Previous ETA studies with SIMAAC[5,6] have shown that in simultaneous multi-element analysis, the choice of atomisation temperature must be biased in favour of efficient volatilisation of the less volatile elements. Although an intelligent guess at the required atomisation temperature for probe - SIMAAC could be made on the basis of previous experience, the nature of probe atomisation is sufficiently different from wall and platform atomisation to merit detailed evaluation. Hence, the effect of atomiser temperature on the magnitude and precision of probe - SIMAAC signals was investigated for a range of elements of varying volatility. The temperature range studied was 1500–3000 °C. The effect of atomiser temperature was considered for three groups of elements categorised, depending on their atom appearance temperature, as volatile, medium volatile or involatile.

The change in peak-height and peak-area signals with atomisation temperature for nine elements (three elements per category) is illustrated in Figs. 2–4. For the probe system used in this work, the atomiser temperature determines the value of the gas-phase temperature within the atomiser and also the heating rate of the atomisation surface, the probe. It might, therefore, be expected that as the atomiser temperature is increased, the probe heating rate would also increase and cause a corresponding increase in the value of the peak-height absorbance. In addition, provided the total number of atoms produced during the atomisation stage is unaltered, the associated peak area values should be similar at all temperatures. However, this hypothesis is not consistent with the experimental results obtained for the different categories of elements as illustrated in Figs. 2–4. For most elements the variations from the expected trends can be related to a decrease or increase in the total number of atoms detected as the atomiser temperature is increased. In this respect changes in peak-height values are consistent with the observed trends for peak-area measurements.

For the volatile elements (Cd, Pb, Zn; Fig. 2) the peak-area sensitivity decreases by a factor of two over the temperature

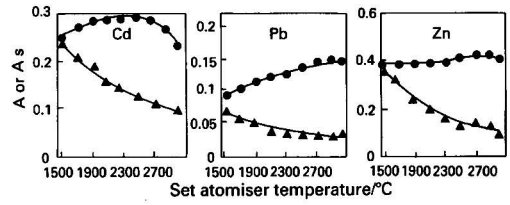

Fig. 2. Atomisation stage temperature optimisation graphs for cadmium, lead and zinc; plots of peak-height (●) and peak-area (▲) probe atomisation signals against set atomiser tube temperature (10 μl of 200 μg l^{-1} Cd, 200 μg l^{-1} Pb and 100 μg l^{-1} Zn)

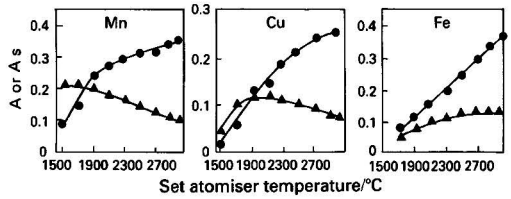

Fig. 3. Atomisation stage temperature optimisation graphs for manganese, copper and iron; plots of peak-height (●) and peak-area (▲) probe atomisation signals against set atomiser temperature (10 μl of 100 μg l^{-1} Mn, 100 μg l^{-1} Cu and 100 μg l^{-1} Fe)

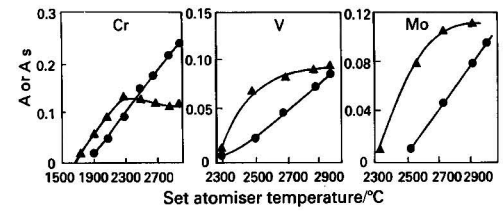

Fig. 4. Atomisation stage temperature optimisation graphs for chromium, vanadium and molybdenum; plots of peak-height (●) and peak-area (▲) probe atomisation signals against set atomiser tube temperature (10 μl of 100 μg l^{-1} Cr, 400 μg l^{-1} V and 400 μg l^{-1} Mo)

range 1500–3000 °C. This loss in sensitivity is probably due to increased diffusional loss of atoms at higher temperatures. The decrease in the peak-area signal for the volatile elements is greater than observed for tube-wall atomisation because the existence of a slot on the tube wall reduces the residence time of atoms, especially at elevated temperatures.[21] With peak-height measurements, the increase in atomisation rate counteracts the effect of diffusional loss at higher atomiser temperatures, with the result that the peak-height sensitivity for Cd, Pb and Zn does not change greatly over the temperature range studied. It is interesting that the most volatile of the three elements, cadmium, shows a slight decrease in the peak-height signal above 2700 °C, whereas the peak-height signal for lead continues to rise slightly up to 3000 °C.

The temperature optimisation graphs for the medium volatile elements (Cu, Fe, Mn) are shown in Fig. 3. Although the shapes of the peak-height and peak-area graphs are different to those obtained for the volatile elements, the trends observed can be explained in a similar manner. For each of the medium volatile elements there is a temperature range over which the peak-area sensitivity is approximately constant. Within this range, peak-height signal increases are expected, owing to the influence of temperature on the atomisation rate. For manganese the levelling-off of the peak-height sensitivity at higher temperatures is consistent with the decrease in the peak-area sensitivity, which is presumably due to greater diffusional loss of atoms. The peak-area graphs for copper and iron show that in the

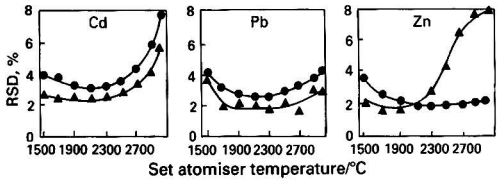

Fig. 5. Precision of peak-height (●) and peak-area (▲) probe atomisation signals against set atomiser tube temperature for cadmium, lead and zinc (see Fig. 2 for concentrations)

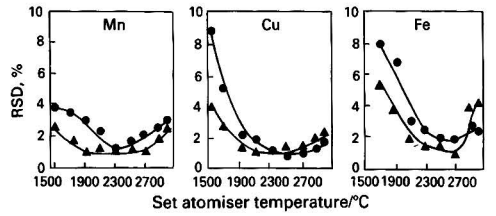

Fig. 6. Precision of peak-height (●) and peak-area (▲) probe atomisation signals against set atomiser tube temperature for manganese, copper and iron (see Fig. 3 for concentrations)

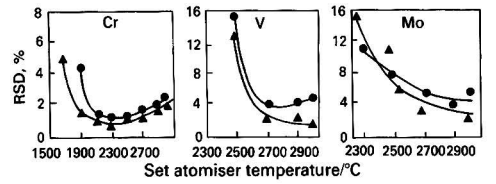

Fig. 7. Precision of peak-height (●) and peak-area (▲) probe atomisation signals against set atomiser tube temperature for chromium, vanadium and molybdenum (see Fig. 4 for concentrations)

Table 2. Comparison of peak time values (t_{peak}) for tube-wall and probe atomisation with a Perkin-Elmer HGA-500 atomiser. Atomisation temperature 2700 °C at zero ramp

Element	Peak time/ms	
	Wall	Probe
Cd	250	110
Zn	360	125
Pb	430	142
Mn	589	178
Cu	660	214
Fe	710	214
Ni	1000	250
Al	780	285
Cr	890	285
Co	820	321
Ca	1080	390
V	1320	460
Mo	1320	860

temperature range 1500–2300 °C increased atom formation is obtained, which also contributes to the general rise in peak-height sensitivity as temperature is increased. The improvement in the peak-area signal for copper and iron may be due to more efficient evaporation of Cu and Fe salts from the probe, but may also indicate an enhancement of atomisation efficiency due to the greater dissociation of vapour-phase molecules at higher temperatures. As the atomiser temperature is increased, the partial pressure of oxygen that exists in the furnace decreases[26] and so dissociation of metal oxide molecules will be greater at higher temperatures.

Table 3. Comparison of peak-height and peak-area detection limits for SIMAAC with probe atomisation. Compromise atomisation temperature of 2700 °C. Detection limits based on 3σ of the mean of the base-line noise

Element	Detection limit/μg l⁻¹	
	Peak height	Peak area
Cd	0.85	3.20
Zn	0.44	0.84
Pb	1.50	5.60
Mg	0.01	0.04
Mn	0.26	0.51
Cu	0.30	0.30
Fe	0.53	3.90
Co	4.70	5.90
Ni	6.90	9.30
Cr	0.41	0.55
Al	0.70	0.48
Ca	0.10	0.04
Mo	7.50	3.20
V	10.80	3.90
K	8.60	6.30

Table 4. Comparison of best absolute detection limits obtained by SIMAAC at a compromise atomisation temperature of 2700 °C with probe, platform and tube-wall atomisation

Element	Detection limit/pg		
	Probe*	Platform†‡	Tube-wall†§
Ca	0.4	1.8	2.0
Co	47	40	18
Cr	4	10	6
Cu	3	6	2
Fe	5	20	10
Mg	0.1	2.0	0.6
Mn	2.6	6.0	2.0
Mo	32	40	14
Ni	69	60	40
Pb¶	15	67	25
V	29	40	18
Zn	4.4	18.0	8.0

* 10-μl volume; Ca, Mo and V based on peak area, others on peak height.
† 20-μl volume; best values obtained from references 5 and 6.
‡ Ca, Fe, Mg, Ni and Zn based on peak area, others on peak height.
§ Fe based on peak area, others on peak height.
¶ All values obtained in this study.

The temperature optimisation graphs for the involatile elements (Fig. 4) are consistent with the trends portrayed by the medium volatile and volatile elements. The peak-area graphs for each of the three elements studied (Cr, Mo, V) exhibit an initial increase in sensitivity as the atomiser temperature is increased owing to improved volatilisation and atomisation efficiency, but at higher temperatures the gain in atom production is counteracted by an increase in the rate of diffusional loss, and the peak-area sensitivity becomes constant. Throughout the temperature range studied, the peak-height sensitivity for Cr, Mo and V continues to increase.

A comparison of the peak-area temperature optimisation graphs indicates the relative influence that various temperature-dependent processes have on the total number of atoms produced by different elements. Cadmium, lead and zinc are almost totally atomised at comparatively low temperatures. Hence, as the atomiser temperature is increased, the removal of atoms by diffusion is the dominant process, which gives a corresponding decrease in the peak-area sensitivity. In comparison, atom formation at the lower end of the atomisation temperature range studied is incomplete for the less volatile elements. As the atomiser temperature is increased, improvements in volatilisation and atom formation

therefore outweigh the effect of temperature on the diffusional loss of atoms. As a result, there is an increase in the total number of atoms measured until the atomisation efficiency becomes constant and diffusion is again the dominant factor. It is not surprising that the gain in peak-area sensitivity at higher temperatures is more apparent for the involatile elements, as it is Mo, V, etc., which should benefit most from the low partial pressure of oxygen and improved volatilisation efficiency achieved at elevated temperatures with probe atomisation.

On the basis of the temperature optimisation graphs, it is apparently necessary to choose a compromise atomisation temperature of 2700 °C or above. In this temperature range, some loss in sensitivity occurs for peak-area measurements of cadmium, lead and zinc for the reasons mentioned above. However, reasonable precision is obtained for most elements in both peak-height and peak-area measurement modes. For signals in the range 0.05–0.3 A or A s RSD values of ≤4% were obtained, with the exception of zinc by peak area and molybdenum by peak height (Figs. 5–7). The poor precision observed for cadmium and zinc was partly due to the fact that these elements have resonance lines below 280 nm, where the continuum source intensity is low, causing a reduction in the signal to noise ratio. Hence, to achieve a reasonable tube life-time, and obtain acceptable levels of sensitivity and precision, a compromise atomisation temperature of 2700 °C was selected for most of the measurements described in the remainder of this paper. At this temperature, the heating rate of the probe was sufficiently fast to give rapid volatilisation of all elements irrespective of volatility, as illustrated by the t_{peak} values given in Table 2. At 2700 °C the time taken to produce the peak absorbance (t_{peak}) with probe atomisation is a factor of 1.5–4 times shorter than for tube-wall atomisation with zero ramp. The facility to measure t_{peak} values is incorporated as part of the SIMAAC software by means of a simple peak picking routine based on a five-point sliding average filter. There is a small error associated with the t_{peak} values for probe atomisation because the time includes the small but finite period required to move the probe into the atomiser tube by action of the solenoid.

Detection Limits

A comparison of peak-height and peak-area detection limits obtained for probe atomisation at 2700 °C is given in Table 3. The detection limits were computed for individually optimised integration limits, which ranged from 1 s for cadmium to 5 s for molybdenum. As might be expected from the sensitivity and precision data discussed above (Figs. 2–7), peak-height detection limits were a factor of 2–4 better than peak-area detection limits for the volatile and medium volatile elements. The peak-area detection limits for the less volatile elements were in general a factor of 1.5–2.5 times better than the peak-height values. The best probe atomisation detection limits (peak height or peak area) obtained for various elements with the SIMAAC system are compared in Table 4 with the best tube-wall and platform atomisation values obtained from previous studies.[5,6] As different sample volumes were used for probe and platform/tube-wall measurements, the detection limits are expressed by mass (pg) rather than in concentration terms. For most of the volatile and medium volatile elements, the probe detection limits are better than those for platform atomisation. The improvement in detection limit ranges from a factor of 2 for Cu to a factor of 20 for Mg. The best detection limits obtained for Mo and V are similar for probe and platform atomisation. Surprisingly, the values obtained for Co and Ni with the platform were slightly better than for the probe.

In comparison with tube-wall atomisation, under compromise atomisation conditions, the probe gave better detection limits for five elements (Ca, Fe, Mg, Pb and Zn). Similar detection limits were obtained by probe and tube-wall atomisation for Cr, Cu and Mn. For the more involatile elements, Co, Mo, Ni and V, the best probe atomisation detection limits were generally a factor of 2–3 times poorer than the tube-wall detection limits. It is possible that the poor detection limits obtained for the less volatile elements are due to inefficient volatilisation from the probe and/or higher partial pressure of oxygen in the slotted tube atomiser compared with levels of oxygen that exist at higher temperatures in conventional unslotted tubes as used for tube-wall and platform atomisation. Although the final probe temperature was lower than that of the tube wall, inefficient volatilisation was apparently not a severe problem. As shown in Table 2, the time from the start of atomisation to the peak signal for Mo and V with probe atomisation was less than that for tube-wall atomisation. It is possible, therefore, that the partial pressure of oxygen is a more important factor, and that probe atomisation in an unslotted tube would improve the detection limit for Mo, V, etc. The gain in detection limit obtained for the volatile and medium volatile elements by probe atomisation, coupled with a likely reduction in vapour-phase interference effects for these elements, suggests that the probe system may have clear advantages over platform and tube-wall atomisation in simultaneous multi-element analysis, especially as the loss in detection limit for the involatile elements is not particularly significant. A comparison of vapour-phase interference effects observed for probe, platform and tube-wall atomisation will be reported in a separate publication.[27]

Analysis of Standard Reference Materials

Three NBS Standard Reference Materials (SRMs) were analysed to evaluate the accuracy and precision of probe atomisation when used with the SIMAAC system for simultaneous multi-element analysis. The compromise atomisation conditions given in Table 1 were used for all analyses. The results obtained for the simultaneous determination of Ca, Cu, Fe, K, Mn, Na and Zn in bovine liver, rice flour and wheat flour are given in Tables 5, 6 and 7, respectively. The values given are average results for triplicate digests each analysed in duplicate ($n = 6$).

The bovine liver standard has been analysed on a previous occasion by SIMAAC using a platform atomisation procedure.[6] In comparison, the accuracy and precision of the probe results do not appear to be as good as those of the platform data. The reasons for this are not clear. If chemical interference effects cause a systematic error in the probe values, peak-area concentrations might be expected to exhibit consistently better accuracy and precision than peak-height concentrations, but no obvious trend is discernable from the results obtained for any of the standards. In addition, the degree to which the SIMAAC values agree with the certificate concentration varies for different elements in different samples. For example, the rice flour peak-height and peak-area concentrations for Mn are in excellent agreement with the certificate value, but a greater discrepancy exists for similar Mn concentrations obtained for wheat flour and bovine liver. Also, the K concentration in bovine liver measured by SIMAAC shows better agreement with the certificate value than the K concentrations determined for the other standards. Considering that no ash stage was used, unlike the platform atomisation study, the over-all agreement between the probe - SIMAAC and certificate concentrations is reasonable.

Conclusions

It was initally suggested that the fundamental advantages of probe atomisation should give better analytical conditions for simultaneous multi-element analysis than either tube-wall or platform atomisation. This has not been entirely vindicated by the present study. In comparison with previous analyses,[5] the

Table 5. SIMAAC analysis of NBS bovine liver (SRM 1577)

	Concentration/μg g⁻¹			
			Probe atomisation	
Element	Certified value	Platform atomisation[6]	Peak height	Peak area
Ca	124 ± 6	123 ± 5	134 ± 21	129 ± 12
Cu	193 ± 10	190 ± 2	169 ± 7	184 ± 6
Fe	268 ± 8	234 ± 6	310 ± 33	300 ± 31
K	9700 ± 600	9800 ± 80	9791 ± 235	—*
Mn	10.3 ± 1.0	9.44 ± 1.16	9.2 ± 1.1	11.4 ± 3.7
Na	2430 ± 130	—†	2346 ± 300	2462 ± 502
Zn	130 ± 13	120 ± 12	93 ± 17	106 ± 31

* Not reported.
† Concentration not determined owing to end-cone contamination.

Table 6. SIMAAC analysis of NBS rice flour (SRM 1568)

	Concentration/μg g⁻¹		
		Probe atomisation	
Element	Certified value	Peak height	Peak area
Ca	140 ± 20	162 ± 10	136 ± 5.0
Cu	2.2 ± 0.3	1.9 ± 0.2	2.8 ± 0.3
K	1120 ± 20	995 ± 48	—*
Mn	20.1 ± 0.4	20.8 ± 0.4	21.0 ± 0.4
Zn	19.4 ± 1.0	17.3 ± 7.2	—*

* Concentration not determined owing to end-cone contamination.

Table 7. SIMAAC analysis of NBS wheat flour (SRM 1567)

	Concentration/μg g⁻¹		
		Probe atomisation	
Element	Certified value	Peak height	Peak area
Ca	190 ± 10	217 ± 9	—*
Cu	2.0 ± 0.3	1.6 ± 0.3	2.6 ± 1.0
Fe	18.3 ± 1.0	16.7 ± 3.8	23.6 ± 3.9
K	1360 ± 40	1061 ± 114	—*
Mn	8.5 ± 0.5	5.7 ± 0.8	9.6 ± 3.1
Zn	10.6 ± 1.0	14.8 ± 4.2	—*

* Concentration not determined owing to end-cone contamination.

accuracy of the probe procedure is better than that for tube-wall atomisation, presumably owing to a reduction in chemical interference effects, but the detection limits obtained for the involatile elements are worse, although only by a factor of two. The t_{peak} values measured for probe AAS signals generated at 2700 °C suggested that satisfactory vaporisation rates were achieved for all the elements studied. It is possible, therefore, that the poorer detection limits obtained for the involatile elements may have been due to the existence of a higher partial pressure of oxygen in the slotted cuvette compared with the unslotted cuvette as used for tube-wall atomisation. Alternatively, the rate of diffusion of atoms out of the slot may simply have a greater influence on the signals for the involatile elements. The magnitude of an AAS signal depends on the relative rates of atom supply and atom loss. The rate of diffusion of atoms out of the slot will probably be greater than along the length of a tube without a slot. It is possible, therefore, that the rate of atom loss relative to the rate of supply is greater for volatile elements with the slotted tube.

For all but two of the elements studied, the probe gives better detection limits than platform atomisation, but the precision and accuracy of the probe results are inferior for some elements. The poorer precision of the probe - SIMAAC procedure may be due to spreading of the droplet along the stem of the probe during drying. The variation in the accuracy of the probe - SIMAAC results may reflect differences in the degree of chemical interference caused by different sample matrices. With platform atomisation, it is known that temporal separation of background and analyte signals can be achieved, which corresponds to a difference in the vaporisation time of the matrix and analyte. As sample vaporisation rates are much faster with the probe, simultaneous evaporation of the matrix and analyte is more likely. Although the conditions of probe atomisation are thermally more favourable than platform atomisation, the actual concentration of the matrix vapour that exists in the atomiser at the time of analyte measurement is thus much greater. It is therefore possible that for some elements in certain samples, rapid volatilisation from the probe may cause a larger chemical interference than expected. It is clearly necessary to investigate the degree to which different halide salts cause vapour-phase chemical interference effects for probe, platform and tube-wall atomisation under the compromise conditions required for simultaneous multi-element analysis, and this is the subject of a future paper.[27] The findings of the current study suggest that better analytical performance could be achieved with probe atomisation if an unslotted tube is used and if greater control of the probe heating rate is available. Both topics are at present under investigation.

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Determination of the Absolute Mass of an Analyte by Flame Atomic-absorption Spectrometry with Discrete Nebulisation

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The absolute mass of a certain metal in a sample was directly measured by flame atomic-absorption spectrometry with discrete nebulisation by injecting the total solution volume obtained after sample decomposition. A linear calibration graph of peak area *versus* absolute mass was easily obtained with different volumes of one standard solution. The method was applied to the determination of copper or manganese in biological standard samples of 1–10 mg (NBS SRM 1570 spinach, 1573 tomato leaves, 1575 pine needles and 1577 bovine liver). The analytical results agreed well with the certified values.

Keywords: *Atomic-absorption spectrometry; discrete nebulisation; absolute mass method; biological standard samples*

Flame atomic-absorption spectrometry with discrete nebulisation has been studied by many workers and applied to practical analyses.[1] In our previous work,[2–5] peak-area measurement with an automatically triggered digital integrator was applied successfully to analyses of many elements in various samples. Although the peak height increases with increasing metal concentration, irrespective of the injection volume, the peak area increases with increasing absolute mass of a metal in the solution injected.

This paper describes the direct measurement of the absolute mass of copper or manganese in biological standard samples of 1–10 mg by injecting the total solution obtained after sample decomposition. A simple means of obtaining a linear calibration graph over a wide range using only one standard solution is also suggested.

Experimental

Apparatus

A Hitachi Model 518 atomic-absorption spectrometer was used with an automatically triggered digital integrator[6] and a small PTFE funnel coupled directly to the nebuliser capillary. The absorption signal was simultaneously recorded on a strip-chart recorder. All measurements were carried out under the optimum operating conditions reported previously.[5] The sample solution was injected into the funnel with a micropipette.

Reagents

A copper or manganese stock solution (2000 μg g^{-1}, 0.5 mol dm^{-3} HNO_3) was prepared by dissolving copper metal (99.999%, Mitsuwa Pure Chemicals) or manganese dioxide (Specpure grade, Johnson Matthey Chemicals) in concentrated nitric acid and diluting with water by mass. Working solutions were prepared by diluting the stock solutions by mass to appropriate concentrations.

Doubly distilled water from an all-glass still was used throughout. Acids of Super Special grade from Wako Pure Chemicals were used without further purification.

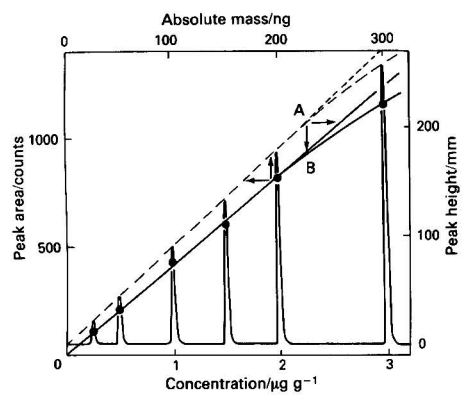

Fig. 1. Calibration graphs obtained with a constant volume (100 μl) of metal solutions of varying concentration. A, Peak height *vs.* manganese concentration; and B, peak area *vs.* absolute mass of manganese

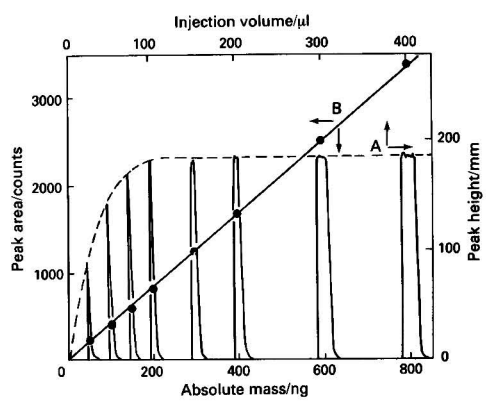

Fig. 2. Calibration graph obtained with different volumes of a constant concentration (2 μg g^{-1}) of metal solution. A, Peak height *vs.* injection volume; and B, peak area *vs.* absolute mass of manganese

Table 1. Recovery of metals from injections of standard solutions

Metal	Standard solution/ ng g^{-1}	Injection volume/ µl	Calculated mass/ ng	Found/ ng	Recovery, %	Calibration: Concentration/ µg g^{-1}	Calibration: Volume/ µl
Cu	49.3	1000	49.3	49.9	101	0.500	0–200
	20.1	500	10.1	10.5	104	1.00	0–200
		1000	20.1	20.1	100		
	201.7	500	100.8	100	99.2		
		1000	201.7	204	101		
Mn	19.2	500	9.6	10.0	104	1.00	0–200
	50.3	1000	50.3	49.6	98.6	0.500	0–300
	150.3	1000	150.3	147.7	98.3		
Mn	50.3	500	25.2	25.3	100	0–0.200	100
		1000	50.3	48.9	97.2		
	150.3	500	75.2	73.5	97.8		
		1000	150.3	150.2	99.9		

Table 2. Analytical results for manganese in NBS SRM 1577 bovine liver

Sample mass/ mg	Final volume*/ ml	Found/ ng	Content/ µg g^{-1}	Calibration: Concentration/ µg g^{-1}	Calibration: Volume/ µl
12.0	~1	128	10.6	1.00	0–200
11.3	~1	116	10.3		
4.899	~0.1	53.2	10.9	0.591	0–300
5.218	~0.3	53.0	10.2		
4.957	~1	51.7	10.4		
0.851	~1	8.69	10.2	0.200	0–200
0.985	~1	10.3	10.4		
0.910	~1	9.31	10.2		
4.745	~0.1	49.7	10.5	0–2.00	100
5.235	~0.1	53.7	10.3		
4.935	~0.3	49.9	10.1		
5.117	~0.3	53.4	10.4		
5.765	~1	57.2	9.9		
Average content			10.3 ± 0.2		
Certified value			10.3 ± 1.0		

* Estimated value.

Table 3. Analytical results for copper in NBS Standard Reference Materials

Sample	Sample mass/ mg	Final volume*/ ml	Found/ ng	Content/ µg g^{-1}	Calibration: Concentration/ µg g^{-1}	Calibration: Volume/ µl
Spinach SRM 1570	10.9	~1	139	12.7	1.00	0–200
	10.2	~1	125	12.3		
	9.9	~1	119	12.0		
	2.349	~0.6	29.0	12.3	0.500	0–200
	1.187	~0.6	14.1	11.9		
	1.185	~0.6	14.3	12.2		
Average content				12.2 ± 0.3		
Certified value				12 ± 1		
Tomato leaves SRM 1573	11.2	~1	128	11.4	1.00	0–200
	10.1	~1	112	11.1		
	10.4	~1	118	11.4		
	2.877	~0.6	31.7	11.0	0.500	0–200
	1.031	~0.6	11.8	11.4		
	1.111	~0.6	13.0	11.1		
Average content				11.2 ± 0.2		
Certified value				11 ± 1		
Pine needles SRM 1575	13.3	~1	43.7	3.3	0.500	0–200
	10.3	~1	45.1	4.4		
	13.4	~1	46.7	3.3		
Average content				3.7		
Certified value				3 ± 0.3		

* Estimated value.

Sample Decomposition

About 1–10 mg of sample and 200 μl of acid mixture were placed in a small PTFE vessel with snap-cap (3.8 ml, N-3, San-ai Kagaku). The acid mixture contained nitric, hydrochloric and perchloric acids (10 + 1 + 1) for digestion of bovine liver and nitric, hydrochloric, hydrofluoric and perchloric acids (10 + 1 + 1 + 1) for other samples. After the small vessel had been placed in a sealed PTFE bomb (23 ml, N-25, San-ai Kagaku), the sample was decomposed by heating at 90 °C for 2 h and then at 140 °C for 2 h in a drying oven.[7,8] The decomposed sample solution was evaporated nearly to dryness and then the residue was dissolved in less than 1 ml of 0.1 mol dm^{-3} perchloric acid in the same vessel. It was not necessary to measure the mass or volume of the final solution because the total volume of the sample solution was injected into the flame for AAS measurement.

Results and Discussion

Peak Area and Absolute Amount of Metal

Using a constant volume, both peak height and peak area are proportional to the metal concentration in the lower range (Fig. 1). At higher concentrations, the peak height and area usually fall below the straight line, as shown in Fig. 1. It should be noted in Fig. 1 that the bottom scale of concentration is converted into the top scale of absolute mass (concentration × injection volume). At constant concentration, a linear relationship between peak area and injection volume holds, over a wide range, while the peak height is unchanged for injection volumes of 100 μl or above (Fig. 2). In Fig. 2, the top scale of injection volume is also converted into the bottom scale of absolute mass. Hence the peak area is proportional to the absolute mass present in the injected solution.[2,5]

The calibration graph of peak area *versus* absolute mass can be obtained in two ways: (1) constant volume of standard solutions containing different metal concentrations (conventional method, Fig. 1, B) or (2) different volumes of a solution of constant concentration (present method, Fig. 2, B). The latter is simple and convenient because only one standard solution is required and the calibration graph is linear over a wide range.

Application to Standard Samples

The recovery of the absolute mass of copper or manganese was examined using standard solutions of various concentrations and injection volumes. The results are given in Table 1 with the concentration and injection volume for calibration. The absolute mass of each metal in the solution injected was found directly from the calibration graph and the recovery was obtained by dividing the found value by the calculated value of concentration × injection volume. The conventional calibration method was also tried for comparison. The recovery observed is satisfactory, irrespective of the concentration, injection volume and resulting absolute mass. Hence, two methods for calibration are available.

The proposed method was applied to the determination of manganese in NBS SRM 1577 bovine liver and of copper in NBS SRM 1570 spinach, 1573 tomato leaves and 1575 pine needles. The results are shown in Tables 2 and 3. The content of each metal in the original sample was calculated directly from the measured absolute mass and the mass of the sample aliquot. Manganese in bovine liver was also determined using the conventional calibration procedure. All results were in good agreement with the certified values and their relative standard deviations were very small.

In conclusion, the proposed absolute mass method, *i.e.*, measurement of the peak area of the absorption signal resulting from the injection of the total volume of sample solution and calibration using only one standard solution, were found to be convenient for the determination of copper and manganese in a small mass of investigated sample.

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Carbonisation Technique for Pre-treatment of Biological Materials in X-ray Fluorescence Spectrometry

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The carbonisation of biological material is proposed as a technique for facilitating the destruction of samples prior to the determination of elements by X-ray fluorescence (XRF) spectrometry. Biological materials heated at 300 °C for 1–2 h are carbonised and easily powdered to fine particles by using an agate mortar and pestle. Losses of inorganic elements from the samples during carbonisation at 300 °C for 1 h were less than 5% for sodium, magnesium, aluminium, chlorine, potassium, calcium, vanadium, chromium, manganese, cobalt, nickel, copper, zinc, bromine, rubidium and cadmium, but more than 30% for selenium and about 100% for mercury. The carbon remaining in the carbonised samples acts as a binder for the sample when discs are made.

Keywords: *Carbonisation; biological material; dry ashing; X-ray fluorescence spectrometry*

X-ray fluorescence (XRF) spectrometry has become one of the most widely used techniques for the elemental analysis of biological samples. However, a pre-treatment such as powdering, dry ashing or wet digestion is required for the destruction of the samples prior to the determination of the elements by XRF spectrometry, unless the samples consist of fine particles or are thin initially.[1]

Accordingly, various powdering equipment such as a mortar, blender and mill using an agate mortar,[2] a stainless-steel vial,[3] a steel grinding vial covered with hard chrome plate,[4] and a tungsten carbide-lined barrel[5] has been used for powdering.[6–9] However, the ease with which powdering can be effected differs with samples and the powdering of many biological samples is difficult. For example, powdering of bone, dried muscle, shells and hard plants is problematic and there are also many biological materials, such as rice plants and bamboo leaves, that are difficult to homogenise to fine particles in spite of their soft tissues.

In general, the powdering process is time consuming and suffers from contamination or loss of the elements to be determined. Dry ashing of biological material at a temperature greater than 450 °C for more than half a day is also time consuming and complete dry ashing is usually difficult without chemical aids, although it varies according to the type of sample. Dry ashing may lead to losses of elements such as selenium, arsenic, chromium, manganese, nickel, lead, tin and strontium by volatilisation and by retention on the surfaces of reaction vessels used at high temperatures, and the use of chemical aids may cause contamination.[10–16]

The wet digestion of biological material using various reagents has improved recently, especially with regard to the quality of reagents and, therefore, the risk of contamination from the reagents has decreased; however, this technique is not always suitable for the analysis of biological samples using XRF, it is time consuming and there is a risk of contamination or loss of elements during the complicated treatment.

One of the reasons why biological samples are not usually analysed quantitatively by XRF, in spite of the many merits of this non-destructive technique, is the difficulty of pre-treatment. Therefore, new techniques that permit the destruction of most biological samples without contamination from the reagents, loss of elements or being too time consuming are required in order to facilitate pre-treatment in XRF analysis. The carbonisation technique described in this paper was devised to fulfil these requirements.

Experimental

Materials

Plants and animals including terrestrial and aquatic plants, seaweeds, fish, birds, insects, crustaceans and shellfish (Table 1) were used to evaluate the carbonisation technique. The terrestrial plants were *Alnus hirsuta, Athyrium yokoscene, Cryptomera japonica, Liriodendron tulipifera, Osmonthus ilicifolius, Pinus densiflora, Pleoblastis chino* and *Quercus myrsinaefolia* and the aquatic plants were *Drepanocladus fluitans, Egeria densa, Eichhornia crassipes, Jungermannia vulcanicola, Oryza sativa, Pellia endiviifolia, Phragmites australis, Rhynchostegium riparioides, Scapania undulata* and *Spirodela polyrriza*. The main organic matter in terrestrial and aquatic vascular plants is known to be cellulose.

The seaweeds were *Hizikia fusiforme* and *Gelidium amansii*, whose main organic matter is laminaran. The fish were *Katsuwonus pelamis* and *Pneumatophorus* sp., the muscle and bone being used. Muscle is mainly composed of myosin and actin and bone of calcium phosphate, collagen and mucopolysaccaride protein. Muscle and bone of the bird *Gallus gallus domesticus* were used. The insects used were *Agerastica coelurea, Alaus berus, Anomara cuprea, Apotomopterus japnicus, Leptura ochraceofasciata* and *Xyrotrechus chinensis*, which are mainly composed of screroprotein. The crustaceans were *Neomysis intermedia, Pacifastacus trowbridgii* and *Sergia lucens*, the shells of which are composed of chitin. The shellfish checked were *Corbicula japonica, Meretrix iusoria, Mytilus edulis, Notohaliotis gigantia, Soren strictus* and *Tapes japonica*, the shells of which are composed of calcium carbonate and conchiolin.

Apparatus

A thermostatically controlled muffle furnace (Carbolite, Type ESFZ ELP) was used for carbonisation and dry ashing of biological material.

Most of the carbonised material was powdered manually using an agate mortar and pestle, but some was powdered further using an electrical mortar (Fritsch, laboratory mortar grinder) with an agate vial. The determination of inorganic elements in biological material was performed using a γ-ray spectrometer with a Ge(Li) semiconductor detector after activation in a nuclear reactor, and using an energy-dispersive XRF spectrometer (Ortec TEFA 6111 and Ortec TEFA III). The accuracy of the activation analysis should be about ±5% and the accuracy of the XRF analysis should be less than ±5%. Table 2 shows the nuclear data for activation analysis. The analytical conditions employed for XRF analysis were as follows: Mo anode, Mo filter, anode current 100 μA, anode voltage 45 kV and time 1000 s. The detection limits of XRF analysis (three times the standard deviation) are Cr 0.30, Mn 0.20, Co 0.13, Ni 0.10, Cu 0.08, Zn 0.08, Hg 0.13, As 0.05, Se 0.05, Br 0.05 and Rb 0.05 μg.

The morphological observation of carbonised and powdered samples was carried out using a phase-difference

Table 1. Samples employed for carbonisation and results

Plant*	Animal*
Alnus hirsuta	*Fish:*
Leaves (+); branch (+)	*Katsuwonus pelamis*
Athyrium yokoscene	Muscle (+); bone (+)
Leaves (+); branch (+)	*Pneumatophorus* sp.
Cryptomera japonica	Whole body (+)
Leaves (+); branch (+)	
Drepanocladus fluitans	*Bird:*
Shoot (+)	*Gallus gallus domesticus*
Egeria densa (Brazilian elodea)	Muscle (+); bone (+);
Leaves and stalk (+)	fat (−)
Eichhornia crassipes	
(water hyacinth)	*Insects:*
Leaves and stalk (+)	*Agerastica coerulea*
Gelidium amansii	Whole body (+)
Thallus (+)	*Alaus berus*
Hizikia fusiforme	Whole body (+)
Thallus (+)	*Anomara cuprea*
Jungermannia vulcanicola	Whole body (+)
Shoot (+)	*Apotomopterus japnics*
Liriodendron tulipifera	Whole body (+)
Leaves (+)	*Leptura ochraceofasciata*
Oryza sativa (rice plant)	Whole body (+)
Leaves (+); stalk (+)	*Xyrotrechus chinensis*
Osmonthus ilicifolius (holly)	Whole body (+)
Leaves (+); branch (+)	
Pellia endiviifolia	*Crustaceans:*
Shoot (+)	*Neomysis intermedia*
Phragmites australis (reed)	Whole body (+)
Leaves (+); stalk (+)	*Pacifastacus trowbridgii*
Pinus densiflora	Whole body (+)
Leaves (+); branch (+)	*Sergia lucens*
Pleoblastis chino (bamboo	Whole body (+)
grass)	
Leaves (+); culm (+)	*Shellfish:*
Quercus myrsinaefolia	*Corbicula japonica*
Leaves (+); branch (+)	Whole body (+)
Rhynchostegium riparioides	*Meretrix iusoria*
Shoot (+)	Whole body (+)
Scapania undulata	*Mytilus edulis*
Shoot (+)	Whole body (+)
Spirodela polyrriza	*Notohaliotis gigantia*
Shoot (+)	Whole body (−)
	Soren strictus
	Whole body (+)
	Tapes japonica
	Whole body (+)

* (+) Samples carbonised and easily powdered using an agate mortar. (−) Samples difficult to carbonise or powder.

microscope and a scanning electron microscope (Hitachi, Type HHS-2R).

Sample Preparation

The biological samples were initially dried at 80 °C for 6–12 h before carbonisation and then carbonised in a muffle furnace in the range 150–450 °C for 15 min to 4 h. The carbonised samples were powdered using an agate mortar and pestle.

Dry ashing was performed on the dried sample at 450 °C for 5 h.

Evaluation of the Loss of Elements

The loss of elements from the samples during carbonisation and dry ashing was evaluated by activation analysis and XRF spectrometry. Activation analysis was performed on the aquatic plants *Jungermannia vulcanicola* and *Pellia endiviifolia* collected from fields and *Egeria densa*, which was cultivated in culture solutions containing cobalt, nickel,

Table 2. Nuclear data for activation analysis

Element	Nuclide	Half-life*	γ-Ray photopeak/keV
Na	^{24}Na	15.02 h	1369, 2754
Mg	^{27}Mg	9.46 m	844, 1014
Al	^{38}Al	2.24 m	757, 1779
Cl	^{38}Cl	37.29 m	1642, 2167
K	^{42}K	12.38 h	1525
Ca	^{49}Ca	8.8 m	3083
V	^{52}V	3.76 m	1434
Cr	^{51}Cr	27.8 d	320
Mn	^{56}Mn	2.58 h	847, 1811, 2113
Co	^{60}Co	5.24 y	1173, 1332
Ni	^{58}Co	71.3 d	810
Cu	^{66}Cu	5.10 m	1039
Zn	^{65}Zn	245 d	1115
As	^{76}As	26.3 h	559, 657
Se	^{75}Se	120 d	136, 265, 280
Br	^{82}Br	35.87 h	554, 619, 777, 828, 1044, 1317, 1474
Rb	^{86}Rb	18.66 d	1077
Cd	^{115}In^{m}	4.5 h–2.3 d	337
	^{115}Cd	2.3 d	528

* h = hours; d = days; m = months; y = years.

arsenic, selenium, bromine and rubidium to enrich their contents in the tissue. The contents of the elements (sodium, magnesium, aluminium, chlorine, potassium, calcium, vanadium, chromium, manganese, cobalt, nickel, zinc, arsenic, selenium, bromine, rubidium and cadmium) in the tissues before and after carbonisation were compared. The losses of elements during the carbonisation procedure was also evaluated using an XRF spectrometer on thin leaves of the floating aquatic plant water hyacinth (*Eichhornia crassipes*), which was cultivated using the same solutions used for *Egeria densa* to enrich the contents of the elements in the tissue. For XRF

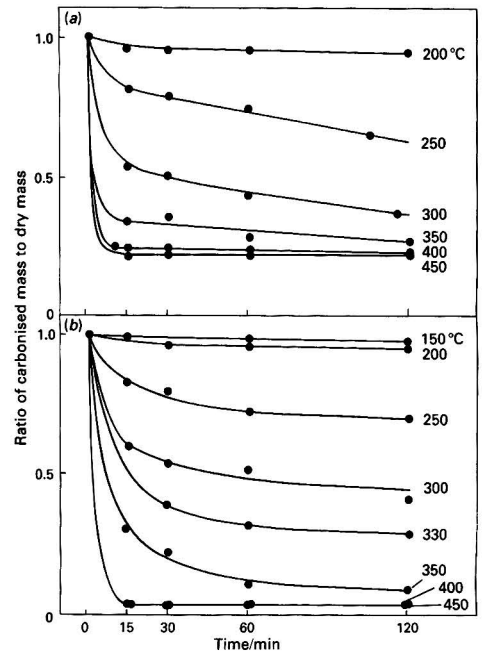

Fig. 1. Time course of the ratio of carbonised mass to dry mass treated at different temperatures for (*a*) rice plant (*Oryza sativa*) and (*b*) bamboo grass (*Pleoblastis chino*)

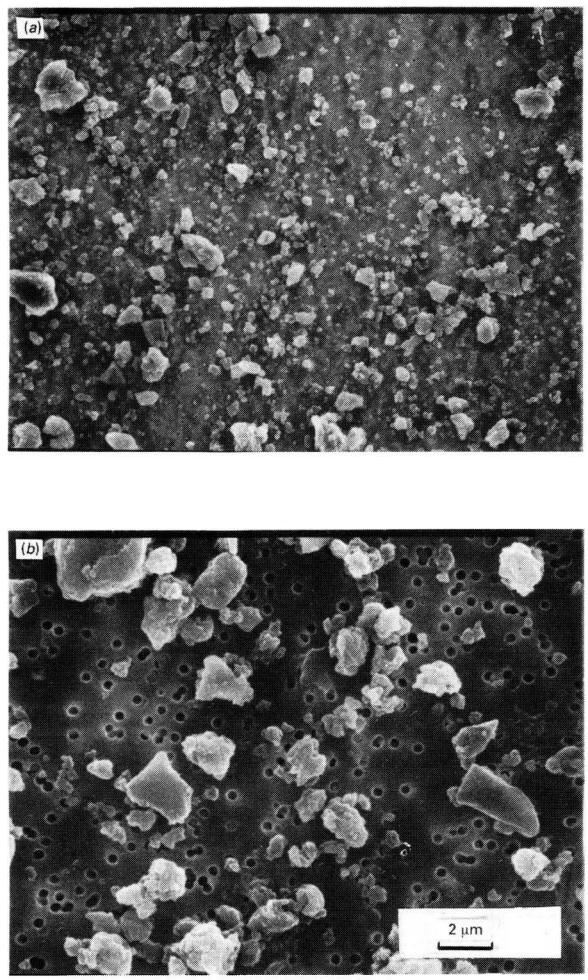

Fig. 2. Photographs of carbonised and powdered rice plant (*Oryza sativa*): (*a*) × 1000; and (*b*) × 5000

[*to face page 1166*

analysis, 5 × 5 mm squares of thin leaves of water hyacinth were cut and analysed. The same samples were then carbonised and re-analysed. The matrix (absorption) effect of the XRF lines (Kα lines) from elements having atomic numbers higher than 24 (chromium) are small theoretically for thin and low-density materials, such as the section of *E. classipes*, which is thin (0.25 mm) and of low density (0.32). The evaluation of the loss of elements was also carried out on the elements in the shoots of the aquatic bryophytes *Jungermannia vulcanicola, Scapania undulata* and *Pellia endiviifolia*, which contain heavy metals in their thin leaves.[17]

Inorganic compounds of cobalt, nickel, arsenic, selenium, bromine and rubidium were not used for the evaluation because the chemical form of such compounds may not be the same as that in biological tissue.

Results and Discussion

Carbonisation

At the beginning of this study, two plants known to be difficult to powder were selected, *viz.*, rice plant (*Oryza sativa*) and bamboo grass (*Pleoblastis chino*). Samples of both were dried and then heated, and the process of carbonisation to ashing was observed.

Isotherms of the ratio of dry mass (D.M.) to carbonised mass (C.M.) against time are given for a range of temperatures in Fig. 1. As can be seen, the ratio C.M./D.M. decreased with increasing temperature and time. Samples heated at 300 °C for 1 h lost 50–60% of their initial mass when carbonised. Further, part of the samples began ashing at higher temperatures after the carbonisation (350 °C for 2 h or 400 °C for 15 min). The carbonised rice plant and bamboo grass were easily powdered to fine particles manually using an agate mortar and pestle. The particles of carbonised rice plant obtained ranged from 0.5 μm in diameter to several times this value (Fig. 2).

Comparison of the samples carbonised at temperatures of 150–350 °C showed that treatment at 300 °C for 1–2 h provides good carbonisation conditions, and these were employed for the subsequent carbonisation of the various biological samples.

The results showed that heating of the sample at 300 °C for 1–2 h was effective for carbonisation and powdering of the biological samples using an agate mortar and pestle, which is preferable to a metallic mortar or blender because of the smaller risk of contamination, although there were two exceptional samples. Further, it was also possible to make discs from carbonised and powdered sample without employing a binder. Preparing discs is a technique often used for the determination of elements by XRF spectrometry.

The two exceptions were the shell of the shellfish of awabi (ear shell, *Notohaliotis gigantia*) and fat. Powdering of the shell of awabi with an agate mortar was impossible, in spite of the ease with which other shellfish could be powdered. The probable reason is the low content of chonchiolin in comparison with other shells. Freshwater shellfish have relatively soft shells and contain more conchiolins than marine shellfish, which have hard shells. Fat is troublesome because of boiling and overflowing to form foams during carbonisation, as is well known in dry ashing.

Loss of Elements

The losses of elements from samples during carbonisation and dry ashing obtained by activation analysis and by XRF spectrometry are shown in Table 3. Losses of sodium, magnesium, aluminium, chlorine, potassium, calcium, vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, bromine, rubidium and cadmium were less than about 5%. However, more than 60% of selenium and more than 10% of bromine were lost during dry ashing at 450 °C for 5 h. The mercury in the samples of the aquatic liverworts *Scapania undulata* and *Jungermannia vulcanicola* (more than 1000 p.p.m.), present as a mercury - sulphur compound, was lost completely during carbonisation.[17,18]

The loss of elements from biological materials during dry ashing has been well documented. Gorsuch[12] examined the

Table 3. Losses of inorganic elements during carbonisation and dry ashing. Biological samples [(A) water hyacinth, *Eichornia crassipes*; (B) Brazilian elodea, *Egeria densa*; (C) liverwort, *Jungermannia vulcanicola*; (D) liverwort, *Pellia endiviifolia*; (E) liverwort, *Scapania undulata*] were carbonised (300 °C for 1 h) and dry ashed (450 °C for 5 h). The results are expressed as the ratio *kC/I* [*k* = ratio of dry mass of carbonised (or dry ashed) sample to initial dry mass; *C* = content of element in carbonised (or dry ashed) sample; and *I* = initial content of element]. The ratios obtained by activation analysis are normalised to the ratio *kC/I* for potassium, which is nearly 1.0. The ratio for *P. endiviifolia*, dry ashed, is normalised to the ratio for copper

	Carbonisation								Dry ashing: XRF analysis	
	XRF analysis			Activation analysis						
Element	A	C	E	B	B	B	C	D	A	D
Na				1.0	1.0	1.0		1.0		
Mg								0.98		
Al								1.0		
Cl				1.05	1.0	1.0	1.0	0.96		
K				*1.0*	*1.0*	*1.0*	*1.0*	*1.0*		
Ca				0.99	0.97	0.91	0.92	0.98		
V								0.96		
Cr	0.99				1.0				1.0	
Mn	1.0							0.98		0.99
Co					1.06				1.1	
Ni	0.96							0.96	0.96	
Cu								0.96		*1.0*
Zn	1.0				0.95			1.0	0.95	1.05
As	1.0					0.56		0.98	0.77	
Se	0.71					0.43			0.36	
Br	0.98			0.94	0.99	1.0	0.97	1.0	0.89	
Rb	0.97			1.0	0.86	0.96			0.91	
Cd				0.97	1.0	1.1				
Hg		<0.01	<0.01							
Pb										0.97

loss of lead, mercury, zinc, selenium, arsenic, copper, silver, cadmium, antimony, chromium, molybdenum, strontium and iron; no significant losses were reported for lead, zinc, copper, cobalt, silver, cadmium, antimony, chromium, molybdenum, strontium or iron. Presumably no loss of these elements will occur during carbonisation because of the lower temperature and shorter treatment time used. An International Atomic Energy Agency technical report[16] summarised the losses of elements (silver, aluminium, arsenic, barium, calcium, cadmium, copper, iron, mercury, potassium, manganese, molybdenum, sodium, nickel, rubidium, tin, strontium and zinc) during dry ashing of biological materials; the loss was less than 5% at 450 °C for barium, calcium, cadmium, copper, iron, potassium (420 °C), molybdenum, sodium (420 °C) and zinc. However, losses of greater than 5% were observed for silver, aluminium, arsenic, chromium cobalt, mercury, manganese, nickel, lead and tin, although the extent of the loss differed according to the sample. Our results for carbonisation show no loss (<5%) of aluminium, chromium, cobalt, manganese or nickel, which may reflect the differences in the temperature and time of treatment used. The results indicate the merit of carbonisation rather than dry ashing.

The number of biological samples used in the study of carbonisation and powdering reported here is restricted. Further studies are needed to establish the value of this technique, with details of losses of elements in as wide a range of biological materials as possible. Nevertheless, we consider that these results demonstrate the suitability of the carbonisation technique for the pre-treatment of biological materials in XRF spectrometry. It is simple, uses no reagents and requires low temperatures and a short treatment time in comparison with dry ashing.

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Fluorescence Inner Filtering in Double-pass Cell Configurations

Part 1. Primary Inner Filtering

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Relationships are derived for primary inner filtering in double-pass cells and compared with observed inner filtering. It appears that a significant contribution to single-pass inner filtering is caused by a double-pass component in conventional measurement as a direct result of reflection from cell walls. It is possible to determine qualitatively if inner filtering is present and quantification can be effected over a limited absorbance range. The method described is inexpensive and suitable for automation.

Keywords: *Fluorescence; inner filtering*

Fluorescence spectrometry is one of the most sensitive techniques for the determination of trace constituents in samples and, as a consequence, it has been widely applied in many fields of chemical analysis. The applicability of the technique is further enhanced by the specificity imparted by the use of two specific wavelengths to accomplish the analysis. Unfortunately, numerous interferences are experienced in the analysis of real samples, such as quenching of the sample by other matrix constituents. Another problem associated with the technique is significant absorption of the excitation beam by the sample, which is commonly referred to as primary inner filtering. Two categories of primary inner filtering exist. The first is absorption of significant amounts of excitation energy by the analyte, referred to as self-inner filtering, which can be compensated for by the use of non-linear calibration graphs, mathematical correction of the data or dilution of the sample. The second variety is caused by matrix constituents absorbing excitation radiation, for which no correction can be applied unless the absorbance of the matrix at the excitation wavelength is determined by a separate experiment. Dilution of the sample in this instance is not feasible as the analyte concentration is frequently at or near the detection limit. A second form of inner filtering exists whereby significant amounts of fluoresced radiation are reabsorbed by the sample prior to emerging from the sample cell for subsequent measurement by the detector, a condition often referred to as secondary inner filtering; this will be the subject of a subsequent paper. It is unfortunate that there are no commercial instruments available that automatically diagnose either form of inner filtering and, as a consequence, analytical data often appear in the literature that are in error by orders of magnitude.

Theoretical

In the conventional right-angle fluorescence technique, the fluorescence signal, F, is given by the following relationship:

$$F = P_o \varepsilon b C \phi_f K \quad \ldots \quad (1)$$

where P_o represents the power of the excitation radiation, εbC is the absorbance, A, of the analyte, ϕ_f is the quantum efficiency of the fluorophore and K is the collection efficiency of the spectrometer for the fluoresced radiation. This relationship holds true for all optically dilute solutions ($A < 0.001$) at the observation window defined by the emission monochromator slits [Fig. 1(*a*)]. Under inner filtering conditions the value of P_o entering the cell is diminished to P_o' prior to reaching the observation window by the analyte of other matrix constituents. In simplistic form the corrected fluorescence, F_c, can be calculated using the relationship

$$F_c = f_a F_o$$

where F_o is the observed fluorescence signal and f_a is an absorbance correction factor according to Parker and Barnes[1]:

$$f_a = \frac{2.303 A (X_2 - X_1)}{10^{-AX_1} - 10^{-AX_2}} \quad \ldots \quad (2)$$

where A is the absorbance for the sample over a 1-cm path length, b, and X_1 and X_2 are slit parameters defining the dimension and location of the emission window with respect to where the excitation beam enters the sample [Fig. 1(*a*)]. More sophisticated correction factors have appeared in the literature,[2–7] some of which take into account the fact that the excitation beam is partially reflected through the observation window a second time giving a reflection contribution to P_o'

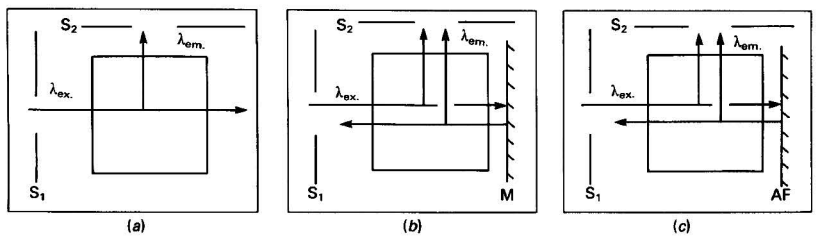

Fig. 1. (*a*) Conventional cell configuration. (*b*) Double-pass configuration associated with the cell compartment of a PE LS-5, where M represents aluminium mirrors machined into the cuvette holder. (*c*) Double-pass configuration constructed from a normal cell compartment, cuvette and aluminium foil (AF). S_1 and S_2 are excitation and emission slits, respectively. See reference 9 for details

that is not adequately corrected for in equation (2)[8] (reference 7 gives an excellent review of these techniques).

In an attempt to increase the signal reaching the detector for increasing sensitivity and/or lowering detection limits, several workers advocate placing a mirror opposite the excitation slit to reflect unabsorbed radiation back through the sample[9–11] and several instruments have appeared with this type of cell compartment, which will be termed "double-pass cell configuration" throughout this paper. This type of mirror placement results in a doubling of the value of P_o of equation (1), at least in theory [Fig. 1(*b*) and (*c*)].

For inner filtered samples in the double-pass configuration, F_o is given by

$$F_o = F_1 + F_2 \quad .. \quad .. \quad .. \quad (3)$$

where F_1 is the fluorescence signal attributable to excitation by P_o' and F_2 is fluorescence from the return pass excitation of power P. An analogous correction expression, f_a', can be derived from equation (2) using new values of X_1 and X_2 that reflect passage of the excitation beam completely through the sample then back to the positions $X_1' = [1 + (1 - X_2)]$ and $X_2' = [1 + (1 - X_1)]$:

$$f_a' = \frac{2.303\,A\,(X_2' - X_1')}{10^{-AX_1'} - 10^{-AX_2'}} \quad .. \quad .. \quad (4)$$

A combination of equations (2), (3) and (4) gives the theoretical inner filtering correction for double-pass cell configurations:

$$F_c = F_1 f_a + F_2 f_a' \quad .. \quad .. \quad .. \quad (5)$$

The difference between the power of the excitation beam for the first- and second-pass fluorescence is the amount of radiation absorbed between the centre of the window for F_1 and the centre of the window for F_2. Assuming the quantity $X_2 - X_1$ to be small compared with b (typically 1.0 cm) and that the window is centred in the middle of the cuvette, then $F_1 \propto P_o'$ and $F_2 \propto P$ for a path length of 1.0 cm. An interesting consequence of this is then

$$A = \log (F_1/F_2) \quad .. \quad .. \quad .. \quad (6)$$

which is useful in quantifying the inner filtering effects attributable to double-pass cell configurations.

Relating Theory to Practice

It should be evident that the preceding discussion largely ignores the following practical considerations, which will be treated only qualitatively in this paper: (*i*) reflections from the rear window of the cuvette adding to the F_1 signal at the expense of F_2 unless otherwise diagnosed by independent experiments, R_c; (*ii*) loss in transmission (and also diffraction) through the rear cuvette wall both prior to and after reflection off the mirror, T_c; and (*iii*) less than 100% efficient reflectivity of the mirror, R_m.

Under non-inner filter conditions, these cumulative effects will have the following effect on equation (5) assuming, for example, a 10% reflection off the rear window of the cuvette, and a 40% loss in excitation power to F_2 due to $[(1 - R_c)T_cR_m]$. The resulting modification of equation (3) is as follows:

$$F_o = (1 + R_c)F_1 + [(1 - R_c)T_cR_m]F_2 \quad .. \quad (7)$$

where the coefficient for F_1 includes the contribution of F_2 in F_1 from reflections off the rear cuvette wall and the coefficient for F_2 represents losses from (*i*)–(*iii*) above. Under ideal non-inner filtered conditions $F_1 = F_2$ and hence a substitution of this condition and equation (7) into equation (6), together with the example values above, yields an apparent absorbance of *ca.* 0.3, which is in agreement with previously published observations for fluorescence signal enhancements using double-pass cell configurations.[9] However, under inner filter conditions this same set of observations substituted into equation (5) yields

Table 1. Inner filter effect corrections

A	f_a	f_a'
0.001	1.00	1.00
0.01	1.01	1.04
0.10	1.12	1.41
0.50	1.77	5.61
1.00	3.15	31.5
2.00	9.78	979

$$F_c = [F_1 f_a + R_c F_2 f_a'] + [(1 - R_c)T_cR_mF_2f_a'] \quad (8)$$

which is considerably more complicated to evaluate owing to difficulties in obtaining exact values for R_c, T_c and R_m.

Experimentally, F_{total} and F_1 are determinable and F_2 is calculated from

$$F_{total} = F_1 + F_2 \quad .. \quad .. \quad .. \quad (9)$$

under conditions prevailing in equations (7) and (8).

Unfortunately, a rapid set of calculations for f_a and f_a' *versus* absorbance according to equations (2) and (4), assuming a path length of 1.0 cm and a centred slit width of 1.0 mm, generates the data in Table 1. From these calculations at high absorbance it becomes apparent that the correction for F_2 becomes so large that its signal will be lost in the noise associated with F_1 at relatively low absorbance values, leading to significant errors associated with the calculation of F_2. Further, the coefficient for F_1 in equation (7) and its analogue in equation (8) will be a function of absorbance varying between $1 + R_c$ at infinite dilution and 1.0 at high absorbance. A consequence of these considerations is that calculational determination of absorbance will be complicated whereas empirical determination can be readily accomplished, provided that reasonably accurate values for F_2 can be obtained.

Experimental

Apparatus

A Perkin-Elmer Model LS-5 spectrofluorimeter was used to give digital indication of fluorescence. Ultraviolet and visible absorbance readings were made using a Perkin-Elmer Model 330 spectrophotometer or a Pye Unicam Model 6-550 spectrophotometer. All measurements were taken in 1 × 1 cm square quartz fluorescence cells.

Reagents

All chemicals used were of analytical-reagent grade and solvents were of either spectroscopic or HPLC quality. Concentrated stock solutions of acetylsalicylic acid in chloroform and salicylic acid in methanol were prepared and analytical standards were made by serial dilutions of the stock solutions. The matrices for the standards were 1% *V/V* acetic acid in chloroform for acetylsalicylic acid and water for salicylic acid. The preparation of the acetylsalicylic acid samples was similar to the method of preparation for the fluorimetric determination of aspirin in tablets but at higher concentrations.[12,13]

Procedure

The solution to be measured was inserted in the cell compartment and read directly (F_{total}) and also with a flat black absorber placed between the cuvette and mirror opposing the excitation slit in Fig. 1(*b*) (F_1) and with the absorber in the cuvette against the wall opposing the excitation slit (F_1'). Alternatively, for conventional cell compartments, the mirrors may be installed as shown in Fig. 1(*c*) to produce a double-pass system.[9] All readings were taken in the 16.8 s integration mode.

Results

The contribution of R_c is both wavelength and solvent dependent and is approximated by

$$R_c \approx \frac{1-T}{1+T} \quad .. \quad .. \quad .. \quad (10)$$

where T is the transmittance of the solvent with respect to air.[4,8,14] For our cuvette using water at 350 and 295 nm, values of 3.8 and 4.4%, respectively were obtained, and similar values were obtained for the chloroform - acetic acid matrix. A comparison of F_1 with F_1' was also performed, which yielded considerably different estimates of R_c. At an excitation wavelength of 295 nm using optically dilute aqueous salicylic acid, a value of 10.1 ± 1.1% was obtained. A later experiment performed in a similar fashion using an aluminium mirror instead of the black absorber gave the value $R_c \approx$ 1.3%. Unfortunately, the original experiment is of questionable validity owing to leaching of small amounts of dye from the black absorber. This is unfortunate, because this result is more in line with computer models that indicate that R_c is the primary contributor to deviation of the slope from a value of 1.0 for plots of $\log(F_1/F_2)$ *versus* A. The term approximating $R_cT_cR_m$ *versus* wavelength has been reported to be 60–63% for various aluminium mirrors at 295 nm in an earlier paper.[9]

Fig. 2 is the theoretical plot of expected fluorescence emission intensity *versus* concentration from equations (2), (3) and (4) (concentration is arbitrarily defined as $C = A \times 100$ as concentration $\propto A$). Figs. 3 and 4 are plots of actual data obtained for the aspirin system, which are typical plots for highly inner filtered (primary) systems that pertain directly to equation (6).

Discussion

From the curves for F_{obs} in Fig. 2, it is evident that inner filtering in double-pass cell configurations will occur at lower A values than in single-pass systems. A consequence of extremely high values for f_a' is that $F_o = F_1$ for $A > 2.0$ and that the curves become double valued at absorbances between the single-pass and second-pass configuration (if such a configuration existed). Extrapolation of the two line segments in Fig. 3 gives an estimate of where inner filtering becomes important in double-pass cell configurations. For the concentration range where no sample absorbance is observed, using equation (6) the observed absorbance will be approximately constant, reflecting values in equation (8) for R_c, T_c and R_m. The second straight-line segment arises from primary inner filtering and intersects the first line at an actual sample absorbance value of *ca.* 0.06. Using the points on the curve at absorbances below 0.06 determines the constant value within the system as 0.316.

This value is in agreement with the value of 0.333 obtained from the intercept of Fig. 4 using a linear regression of data points having sample absorbances between 0.05 and 0.70. The correlation for this regression was 0.997 with a slope of 0.874, which is markedly different from the theoretical slope of 1.0. A non-linear regression analysis of the same data in the form $y = a + bx + cx^2$ was also performed. The intercept from this plot was 0.338 ± 0.006 with a correlation coefficient of 0.9995, and values of 0.900 and −0.195 for b and c, respectively. The small value of c indicates that the linear regression analysis is sufficient for small absorbance values, although a higher slope is indicated for the linear portion of the graph.

Computer-generated data in the form of equation (7) using appropriate values of R_c, T_c and R_m are given in Table 2, *i.e.*, $[(1 - R_c)T_cR_m] \leqslant (1 - R_c)$. From these data it is apparent that the deviation of the slope of the experimental graphs from the theoretical slope of 1.0 is due entirely to the contribution of the R_c term. The experimental graph, having a slope of 0.90, implies a large value of R_c, which is not confirmed by the

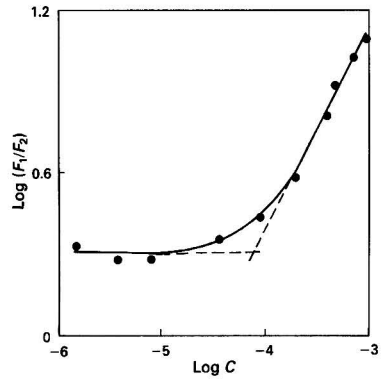

Fig. 3. Graph of $\log(F_1/F_2)$ *vs.* log concentration (C)

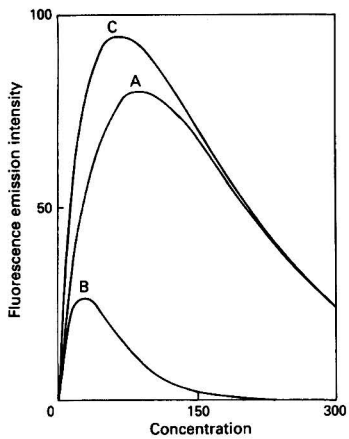

Fig. 2. Theoretical graph of expected fluorescence emission intensity *vs.* concentration for (A) a single-pass system, (B) a second-pass of a double-pass system and (C) a double-pass system

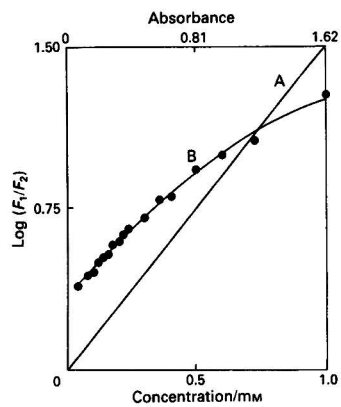

Fig. 4. Graph of $\log(F_1/F_2)$ *vs.* absorbance (or concentration). (A) Theoretical graph where R_c = 0% and $T_c = R_m$ = 100%. (B) Experimental graph corresponding to high concentrations used in the construction of a calibration graph for the fluorimetric determination of aspirin (see references 12 and 13)

Table 2. Summation of data for plots of $\log(F_1/F_2)$ *versus* absorbance

R_c	$(1 - R_c)T_cR_m$	Slope of plot	*y* intercept of plot
0.10*	0.73*	0.98	0.164
0.15	0.73	0.97	0.176
0.20	0.73	0.96	0.189
0.15	0.85	0.9698	0.110
0.15	0.73	0.9697	0.176
0.15	0.50	0.9698	0.341

* Approximate values determined for the chemical systems used in this investigation.

transmission experiments discussed previously. Plots of R_c and $[1 - (1 - R_c)T_cR_m]$ against the *y* intercept indicate that the second expression will have the largest affect on the intercept of a plot of $\log(F_1/F_2)$ against absorbance and that the intercept will approach zero as the former term approaches zero. Neither of these observations is unexpected. However, plots of R_c against the *y* intercept extrapolate to a finite value of T_cR_m, which should be the correct absorbance correction value for the determination of sample absorbance from $\log(F_1/F_2)$. It is important to note that these derivations are based on other assumptions that are not treated in this simplified approach, such as a collimated excitation beam, which experimentally is not true. These other artifacts are still under investigation and are probably the reason why it is actually easier to determine the sample absorbance from empirical graphs than to evaluate it mathematically.

No attempts have been made at this point to explain quantitatively why all plots of $\log (F_1/F_2)$ *versus* A approach a slope of 0.0 at high absorbance values. This fact is also true of similar plots for examples of secondary inner filtering. Qualitatively this effect may be due to non-collimated sources or the fact that at high absorbance values $F_{total} = F_1$, F_2 being negligible compared with the noise in the F_1 signal, making it difficult to obtain reliable values for F_2.

Conclusions

The work reported here clearly demonstrates the feasibility of quantifying sample absorbance that causes primary inner filtering in fluorescence spectrometry. The method presented is the first one that is not based on precise movement of the sample about the cell compartment as in the popular "cell shift methods," which are reviewed in reference 7. This technique should be readily automated by the use of a positioning device and movable mirror located behind the sample cell opposite the excitation source. As most modern spectrofluorimeters are microprocessor or computer controlled, it should be possible to determine the appropriate empirical term, $(1 - R_c)T_cR_m$ *versus* wavelength,[9] which leads to the empirical equation for absorbance determination:

$$A_{sample} = \log(F_1/F_2) - \log(1 - R_c)T_cR_m \quad \ldots (11)$$

and store it for subsequent sample diagnosis at any given excitation wavelength.

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Determination of Aromatic Epoxy Resins and Their Thermal Degradation Products in Air by Infrared Spectrometry

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A quantitative determination of epoxy powder paint and its thermal degradation products in air was carried out by infrared (IR) spectrometry. Using chloroform-*d* as a solvent allowed analysis at 1510 cm^{-1}, where the aromatic absorption is about ten times stronger than at 1610 cm^{-1}. Filter sampling was applied both to epoxy powder and to the high relative molecular mass thermal degradation products of a powder paint. Collection on filters and IR analysis proved to be simple, quick and accurate.

Keywords: *Powder paint analysis; epoxy resin; thermal degradation product; air sampling; infrared spectrometry*

The reaction products of bisphenol-A and epichlorohydrin share 80–90% of the market of epoxy resins. Solid resins with relative molecular masses over 624 ($n = 2$) are used in powder paints. The typical structure of a bisphenol-A epoxy resin is presented in Fig. 1.[1] Although powder painting installations usually have efficient exhaust systems, some powder seems to escape from the painting line to the workroom air. It is known that epoxy resins are sensitising and can cause contact dermatitis[2–4] and lung symptoms.[5,6] Hygienic monitoring necessitates simple methods for the determination of resins in the workplace air.

Relative molecular mass distribution analysis of the resins can be performed by high-performance liquid chromatography (HPLC)[7,8] or gel-permeation chromatography (GPC),[9] but quantification remains a problem as the resins are always mixtures of various oligomers.

IR spectrometric analysis has been presented as a suitable method of analysis in situations where chromatographic separation is not needed or where it is difficult to perform. IR analysis is based on typical absorption at a specific wavenumber by structures of the compound of interest.[10–12]

The aim of our study was to develop methods for the sampling and quantitative analysis of epoxy resin powders in workplace air. Further, we investigated the IR spectrometric determination of the high relative molecular mass fraction of the thermal degradation products of epoxy resins that may be formed in the curing process.

Experimental

Reagents

The reagents used were of analytical-reagent or HPLC grade unless stated otherwise. Epicote 1004, a solid epoxy resin of medium relative molecular mass, was obtained from Shell (The Netherlands) and chloroform-*d* (purum, >99 atom-% D, <0.01% $H_2O + D_2O$) from Fluka AG (Switzerland). The epoxy powder paint was a white amine-curable product obtained from Teknos Ltd. (Finland). The powder was based on Epicote 1004. Throughout the study glass-fibre filters (Whatman GF/A O 37 mm) were extracted with hexane in a Soxhlet apparatus for 16 h, dried in an oven and stored in a desiccator until used.

Apparatus

Liquid chromatography

A Kontron Model 600 liquid chromatograph, equipped with a Rheodyne 7125 injector, a Uvicon 720 UV detector (detection wavelength 279 nm), a Kontron Model 200 programmer and a Hypersil ODS_{18} (5 μm, 200 × 4.0 mm i.d.) column was used in the chromatographic analysis. The rate of elution [using acetonitrile (A) - 1% acetic acid in water (B)] was 1 ml min^{-1}. Gradient elution with 67–57% of B in 8 min was followed by a 2-min isocratic elution after which the concentration of B was reduced linearly to 10% over 5 min.

Infrared spectrometry

A Pye Unicam SP 2000 double-beam infrared spectrophotometer (Pye Unicam Ltd., UK) with a reference cell was employed to quantify the aromatic groups of the epoxy resin and the high relative molecular mass fraction of the thermal degradation products. Sealed KBr cells with a path length of 1 mm were used. As aromatic compounds show strong absorbance at 1510 cm^{-1}, this region was used in the analysis (Fig. 2). The region 1300–1670 cm^{-1} was scanned in 2.5 min.

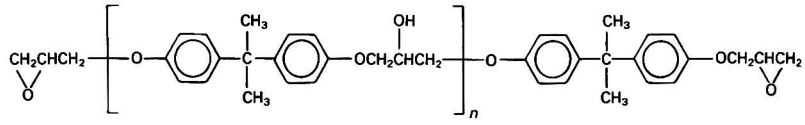

Fig. 1. Structure of bisphenol-A epoxy resins

* To whom correspondence should be addressed.

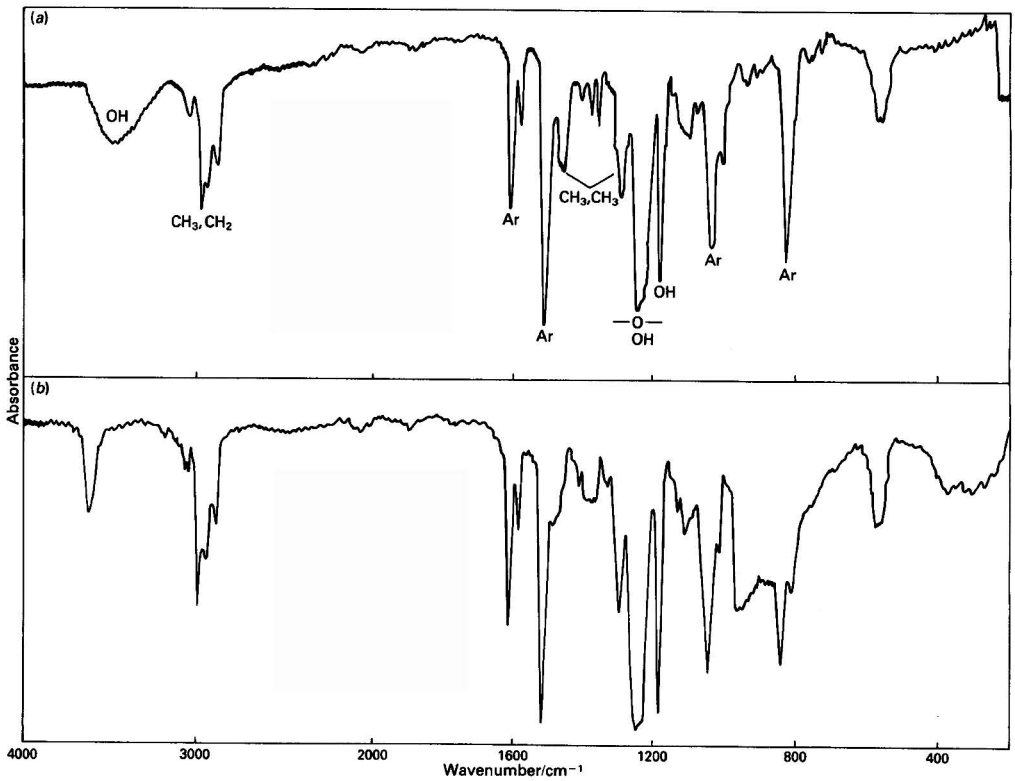

Fig. 2. IR spectrum of a solid epoxy resin (Epicote 1004) in (*a*) potassium bromide and (*b*) chloroform-*d*

Sampling and analysis

For study of recovery, Epicote 1004 was dissolved in $CHCl_3$ and injected on to glass-fibre filters (for amounts see Table 1). The filters were left to dry overnight and then 200 l of air were pumped (Du Pont P 2500) through the filters at the rate of 2 l min^{-1}. Afterwards the filters were placed in 25 ml of $CHCl_3$ for 12–18 h and washed once with 10 ml of $CHCl_3$. The extracts were combined and evaporated to dryness. The residues were dissolved in 0.5 ml of $CDCl_3$.

The epoxy powder paint was thermally degraded in a J-shaped glass tube (inner diameter 4 mm, wall thickness 1 mm, total length 300 mm) inserted into a laboratory oven through a hole. The shorter arm of the tube was completely inside the oven at a temperature of 350 °C. A pump was used to suck air through the degradation tube at the rate of 0.6 l min^{-1} for 30 min. Because aerosols from the thermal degradation of epoxy resin contain phenols,[13] any phenols present were washed from the $CHCl_3$ extracts with three 10-ml portions of 1 N NaOH solution and twice with 10 ml of water. The washed chloroform extracts were dried overnight with anhydrous Na_2SO_4 and evaporated to dryness. $CDCl_3$ (0.5 ml) was added and the sample was analysed by IR spectrometry.

Results and Discussion

The relative molecular mass distribution of epoxy resins varies (Fig. 3), which makes them difficult to quantify in the air samples using HPLC or GPC. Gravimetric analysis cannot be applied because powder paints contain up to 50% inorganic colour pigments and extenders depending on the product. Chloroform ($CHCl_3$) shows strong absorption at 1510 cm^{-1}, a fact that excludes the utilisation of the aromatic absorption in

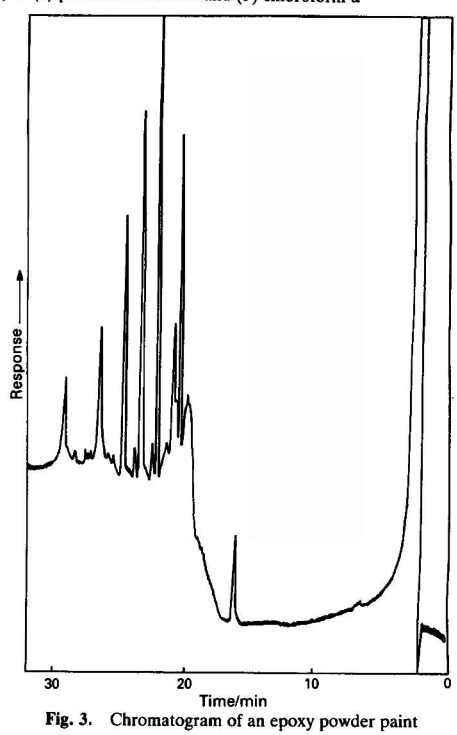

Fig. 3. Chromatogram of an epoxy powder paint

this region. Another alternative is the aromatic absorption at 1610 cm^{-1}. Employment of chloroform-*d* ($CDCl_3$) as a solvent allows the strong absorption at 1510 cm^{-1} to be used (arrows in Fig. 5). For aromatic epoxy resins, the absorption is about ten times stronger at 1510 than at 1610 cm^{-1}. We found the absorbance at 1510 cm^{-1} to be linear in the concentration range 50–740 μg per 0.5 ml, with a detection limit of 50 μg per 0.5 ml (the correlation coefficient of the calibration graph was 0.993, $n = 8$). The reliability of the analysis and the desorption efficiency of the epoxy resin from the glass-fibre filter are presented in Table 1.

The aerosols in the air stream were collected on two filters in series, which were analysed separately. The sampling efficiency for the high relative molecular mass aerosols from the thermal degradation was 100% whereas determination of the desorption efficiency of the degradation products proved impossible because of variable condensation on the walls of the apparatus. Another difficulty was encountered in selecting a suitable calibration substance. The high relative molecular mass fraction, derived from the cracked epoxy network, contains the same aromatic substances as the intact epoxy resin. This enabled us to use pure epoxy resin as a standard for quantification of the high relative molecular mass fraction of the thermal degradation products. HPLC revealed the presence of phenolic compounds [Fig. 4 (*a*)]. These were washed off because they also absorb strongly at 1510 cm^{-1}. The removal efficiency of phenols (100%) was confirmed by HPLC [Fig. 4(*b*)]. The removal of phenols was also observed in the IR spectrum (arrows in Fig. 5), as decreased absorption by hydroxy groups. The remaining absorption was probably due to the secondary aliphatic alcohol groups in the chains. Secondary aliphatic alcohols are not acidic and therefore they are not removed by NaOH extraction.

Table 1. Recovery of epoxy resin from glass-fibre filters

Amount added/μg	Recovery, %	Coefficient of variation, %	Number of samples
72	70	5	3
144	85	6	3
288	97	5	3

Conclusions

This method of sampling and IR analysis of aromatic epoxy resins and their high relative molecular mass thermal degradation products is simple and accurate. The detection limit is 0.2 mg m^{-3} for a 250-l sample (the TLV is 0.5 mg m^{-3}).[14] Linearity and desorption from filters are good at low concentrations.

Financial support from the Finnish Work Environment Fund is gratefully acknowledged. Thanks are also due to Antti Zitting and Professor Pentti Kalliokoski for their kind interest and encouragement.

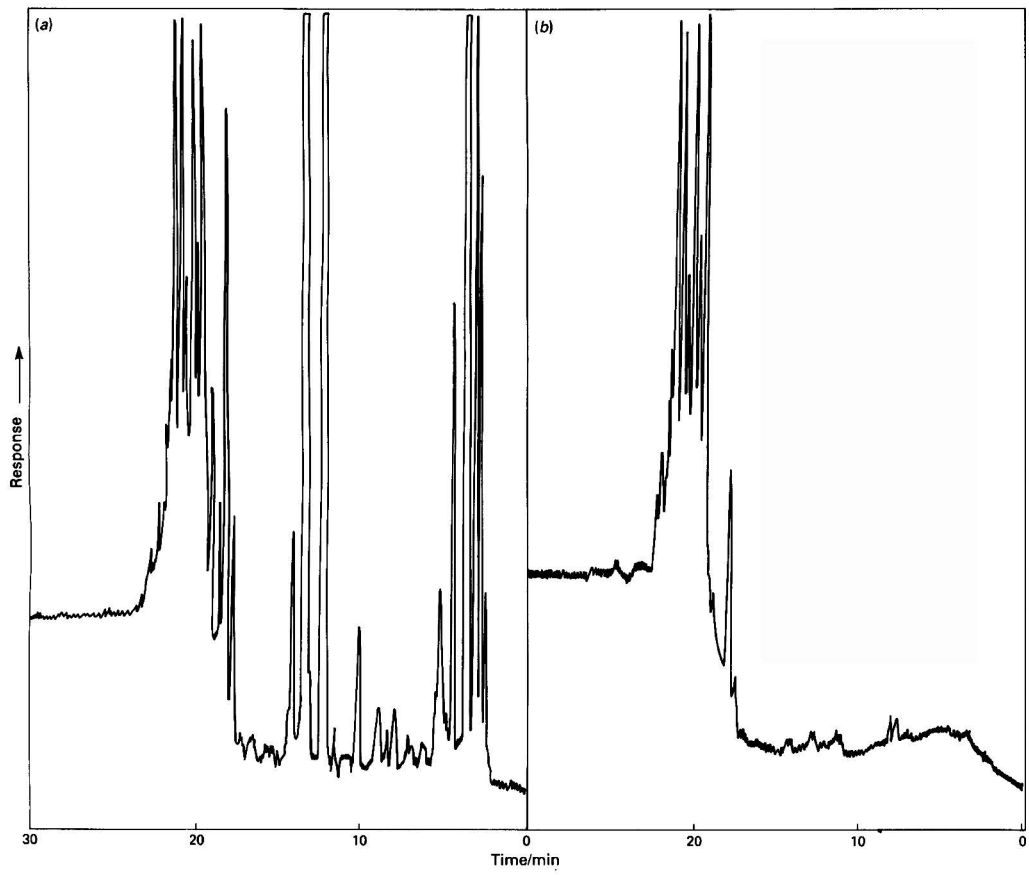

Fig. 4. Chromatogram of aerosols from (*a*) thermally degraded epoxy powder paint and (*b*) a washed aerosol fraction

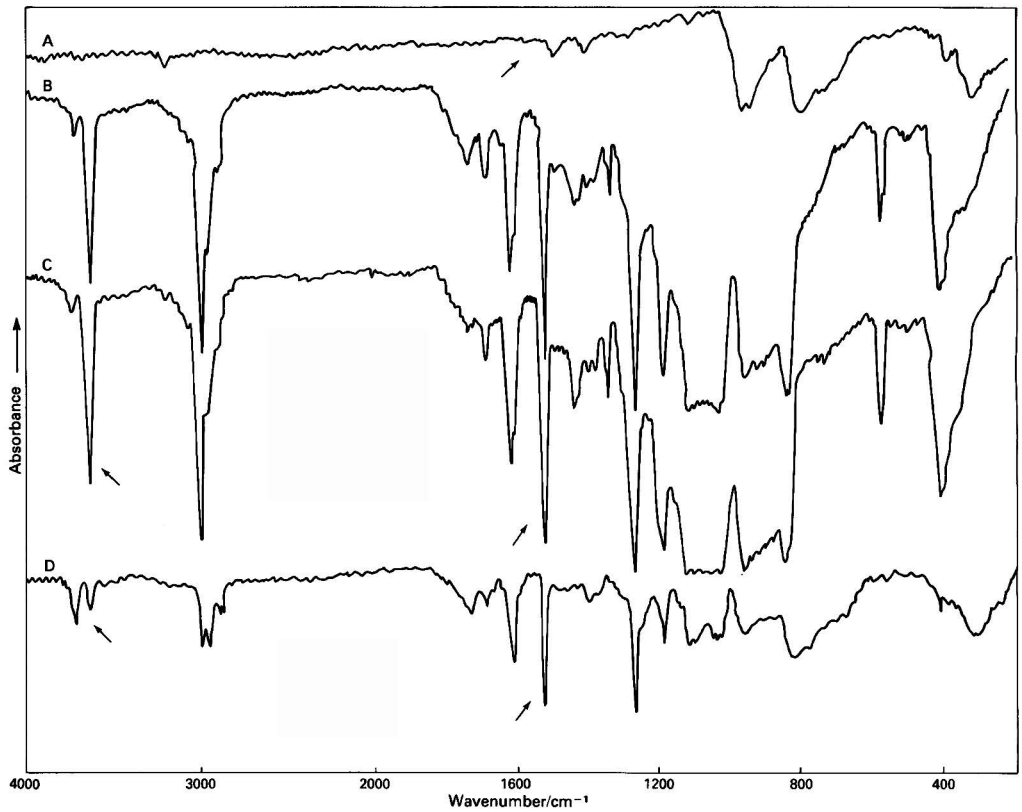

Fig. 5. Effect of the removal of phenols from a filter sample of airborne products of thermally degraded epoxy powder paint. IR spectra: A, chloroform-*d*; B, aerosol fraction before wash; C, part of aerosol fraction extracted by NaOH; and D part of aerosol fraction unextractable by NaOH

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BOOK REVIEWS

Open Tubular Column Gas Chromatography in Environmental Sciences
Francis I. Onushka and Francis W. Karasek. Pp. xiv + 281. Plenum Press. 1984. Price £42.50. ISBN 0 306 41589 5.

Events such as those which occurred recently in Bhopal highlight the human misery that can be inflicted by the release of toxic substances into the environment. Such events also serve to illustrate the problems of detection and identification faced by the analytical chemist in attempting to diagnose the lethal agent. However, the problems faced by the Bhopal chemists were relatively straightforward when one considers the enormous number of organic and inorganic compounds introduced into the environment by today's industrialised society. The analysis of complex mixtures of, for example, polynuclear aromatic hydrocarbons, organochlorine compounds and dioxins calls for methods that are both highly specific and highly sensitive. In this respect capillary gas chromatography has already made a significant contribution and promises to become one of the most important environmental analytical techniques. For these reasons an authoritative text on the subject is most welcome.

The first chapter is principally concerned with the theory of wall-coated open-tubular gas chromatography and in some respects this is the most disappointing part of the book. A beginner to the subject would be wise to skip this chapter, in which many complex equations are introduced with inadequate explanation. Surprisingly, the Golay equation and its practical significance are neglected. There are a number of errors that tend to confuse the situation.

In contrast, chapters 2–5 are informative and contain much sound practical advice, and can be commended to all gas chromatographers contemplating the use of capillary columns. Chapter 2 is concerned with column preparation and it adequately covers materials of construction, pre-treatment processes and coating procedures. The text is accompanied by sufficient theory to give an understanding of how to obtain a useful column. A little more information on the immobilisation of stationary phases could have been included. The next two chapters deal with injection systems and detectors, respectively. In each instance constructional details are accompanied by discussions of the principles involved and practical advice on their correct use. This part of the book is completed by a comprehensive account of sample preparation, which includes all the commonly used isolation and clean-up techniques. A particularly useful aspect is the inclusion of numerous flow charts of procedures recommended for the analysis of the more common environmental pollutants.

The next two chapters deal with peak identification and quantitative analysis. Whereas the latter is perfectly adequate, and can be commended to the beginner, the former may be criticised on the grounds that it is biased towards retention coincidence. Some of the material on mass spectrometry in Chapter 4 could profitably have been transferred to this part of the book and expanded to give a more balanced view.

The final chapter, which occupies a third of the book is concerned with a detailed discussion of the capillary gas chromatography of the full range of organic pollutants. Environmental analysts should find this chapter invaluable as it contains a wealth of detail about specific analytical problems. Further, the chapter is well referenced, as for that matter is the rest of the book.

By today's standards the book is not expensive and at £42.50 represents a sound investment.

M. B. Evans

Laborbücher Chemie. Praxis der Hochleistungs-Flüssigchromatographie. 3. Auflage
Veronika Meyer. Pp. ix + 244. Diesterweg Salle Sauerländer. 1984. Price FF51; DM54. ISBN 3 425 05452 X (Diesterweg); 3 7941 1816 Z (Sauerländer).

The Third Edition of the excellent monograph includes a review of recent developments in HPLC and presents numerous new examples of applications of the technique in the fields of clinical and biological chemistry. As the title suggests, this is a practical work-book for use in smaller chemical and pharmaceutical laboratories in schools, colleges and industry. It is, of course, written in German.

The brief introduction includes definitions of the various HPLC variants and ends with a list of literature sources for further in-depth reading. There then follows a chapter in which important aspects of HPLC theory are presented in sufficient depth to allow the novice practitioner to quickly grasp the fundamentals necessary for critical assessment of a specific analytical problem. The sections of this chapter range from a brief presentation of the chromatographic process, *viz.*, consideration of band broadening, tailing and dead volume, etc., to the optimisation of mobile phases. Each topic is illustrated by reference to practical examples and is accompanied by relevant references.

The main body of the book is given over to a detailed discussion of the components involved in the HPLC process (pumping system, choice of mobile phase, detectors, columns, etc.). Again there is strong emphasis on the practical aspects. Extensive use is made of illustrative line drawings, which are simple and informative. Most of the chemical substances cited are accompanied by their formulae, making the presentation readily understandable even to a reader having a minimal knowledge of German.

Having described the processes by which an HPLC separation can be effected, the author then embarks upon individual expositions of the theory and practice of the most important HPLC—variants currently in common use, *viz.*, adsorption, reversed-phase and liquid - liquid chromatography; chromatography with bound phases; ion-exchange, ion-pair, ion, gel and affinity chromatography. Each technique receives adequate treatment in a volume of this size and academic level, accompanied by a good deal of useful practical detail. Guidance on the choice of an appropriate method is presented, and the author then turns to consideration of elution problems, analytical and preparative HPLC and the more recently developed techniques of micro and capillary HPLC.

The book ends with a series of tables giving useful information on the physical properties and suppliers of the currently available stationary phases classified under the headings of the individual HPLC techniques. The final table lists addresses of suppliers and manufacturers of instruments and associated materials both in the Federal Republic of Germany and in Switzerland.

Presentation of the material and data is clear and well presented. No significant errors were detected. The book represents a useful and relatively inexpensive addition to a laboratory engaged in the practice of HPLC. The liberal inclusion of diagrams and chemical formulae helps to overcome any language barrier that might exist for the potential English-speaking purchaser, although it is suspected that an English edition would find a wide market outside German-speaking Europe.

J. K. Corbett

CRC Handbook of Chromatography. Terpenoids. Volume 1

Carmine J. Coscia. *CRC Series in Chromatography.* Pp. xii + 183. CRC Press. 1984. Price $75 (USA); $86 (Rest of the World). ISBN 0 8493 3004 1.

On the first reading of this book, it is a curious sensation to plunge straight into 32 tables of retention data on gas chromatography, 20 tables on liquid chromatography followed by 36 tables on thin-layer chromatography. This is not the format adopted by some other Editors in this series, where each section is introduced with a description of the class of compound or of the technique.

Coscia describes the gas-chromatographic separations of each of the terpenoid classes in turn, *e.g.*, monoterpenes, sesquiterpenes, diterpenes, triterpenes, sterols, carotenoids, E-vitamers and tocopherols. The chromatographic data are recorded in each table in a standard format, which is a sensible way to permit easy comparisons. However, the retention data are provided in a variety of forms as given in the original work, *i.e.*, absolute retention time, relative elution volumes, methylene units and Kováts retention indices. The literature covered ranges from 1972 to 1980 with a few earlier references where they are necessary.

After immersion in the tables of retention data, it is a relief to reach the second section, which gives a very thorough coverage of the detection reagents used in thin-layer chromatography. The third and last deals adequately with sample preparation and specific detection techniques. This section deals with each of the terpenoid classes that were met in the first section.

This volume is up to the high standard of production associated with CRC publishers and is relatively free from spelling errors and misprints. Some exceptions are the spelling of cardenolides on pp. 132 and 29, where the stationary phase has been omitted. There appears to be no attempt to explain what the abbreviations represent, although the abbreviations for all the colours are given. A good compound index is provided.

The book is very valuable as a compendium of retention characteristics for researchers in the field of terpenoid medicinals, flavours, fragrances and vitamins A, E and K. Although it is expensive, it is well worth ensuring that each research group has access to it.

R. J. Hamilton

CRC Handbook of Atomic Absorption Analysis. Volumes 1 and 2

Asha Varma. Pp. xviii + 510 (Volume 1); xviii + 444 (Volume 2). CRC Press. 1984. Price $99 (USA); $114 (Rest of World) (Volume 1); $89 (USA); $102 (Rest of World) (Volume 2). ISBN 0 8493 2985 X (Volume 1); 0 8493 2986 8 (Volume 2).

This two-volume work is very much in the style of the other titles from this publisher. It is largely a compilation of data, in the form of literature references (with titles), arranged in chronological order.

Volume 1 begins with a brief overview of atomic-absorption spectroscopy including discussions of the theory, instrumentation and methodology, supported by 187 references. The second section, headed "General Instrumentation," is a compilation of 2255 references covering the development of the technique. It is complemented, as are all the reference lists, with an author index and a subject index, which in this instance is divided into "General Instrumentation" and "Applications of Atomic Absorption Spectroscopy." Subsequently, the book divides into a series of sections, each covering a particular group of elements in the Periodic Table. For each element an introductiory section describes its origin, natural chemical forms and chemical preparation. Brief analytical methodologies are given, including a discussion of interferences, the preparation of standard solutions and appropriate instrumental parameters. There then follows the reference list, which for example in the case of Ca numbers 422 entries. The final section of the second volume is devoted to a series of Appendices covering terms and definitions; abstracts, reviews and books; sources; equipment manufacturers; and standards and chemical suppliers.

One can only admire the tenacity of Dr. Varma in obtaining and tabulating the enormous number of entries in this book. There must, however, be reservations about this style of publication in 1985. It competes directly with computerised data bases and, whilst many still prefer a written text ready on the bookshelf, these are out of date at publication and become increasingly so with the passage of time. Further competition is provided by critical reviews such as those contained in the "Annual Reports of Analytical Atomic Spectroscopy" (RSC), which are regularly updated. The "CRC Handbook of Atomic Absorption Analysis" certainly contains a great amount of information; it is perhaps for the individual to decide whether this format is most appropriate to his or her needs.

B. L. Sharp

Techniques in Organic Reaction Kinetics

Petr Zuman and Ramesh Patel. Pp. XII + 340. Wiley–Interscience. 1984. Price £57.25 ISBN 0 471 03556-4.

This monograph was aimed at providing all the necessary background for the planning and execution of investigations of organic reaction mechanisms. The authors assume that readers have a reasonable background in the theory of reaction kinetics, as the text deals with how to obtain and treat data from kinetic studies in order to elucidate mechanisms. Following a brief "Introduction" (pp.5, refs. 2, bibliog. 15), the material is divided into three sections dealing with measurement techniques, analysis of data and finally with the study of fast reactions.

The chapter "Techniques of Reaction Kinetics" (pp.39, refs. 44, bibliog. 3) deals with accuracy of measurement, control of reaction media and measurement of time intervals and of concentration. The latter section, well illustrated by examples, discusses the basic principles and the advantages and disadvantages of various analytical methods for various reactions types.

The major section of the text concerns the "Analysis of Kinetic Data" (pp.201, refs. 331, bibliog. 24) and includes indentification of intermediates, emprical reaction rate equations for simple and complex reactions including parallel, consecutive and competitive reactions and those involving equilibria, determination of reaction order and the evaluation of rate constants. Discussion of the effects of pH and temperature is followed by consideration of the effects of solvent and ionic strength. Semi-empirical and empirical treatments are given of solvent constants including the Kamlet and Taft multi-parameter approach. The chapter concludes with consideration of relationships between structure and reactivity, mainly via the use of the Hammett equation.

The last chapter, "Study of Fast Reactions" (pp.81, refs. 96, bibliog. 28), is a useful summary of material not readily available elsewhere to the advanced undergraduate/young research worker.

This is a detailed and scholarly text of particular interest to experimental physical and physical - organic chemists. It will also be of use to those analytical chemists whose colleagues have the sense to consult them about good analytical methodology prior to collecting kinetic data for organic reaction mechanistic studies.

D. Thorburn Burns

Practical Analytical Electron Microscopy in Materials Science

David B. Williams. Pp. vii + 153. Verlag Chemie International. 1984. Price DM120. ISBN 0 89573 307 2; 3 527 26224 5.

Using the everyday acronyms that beset electron optical studies, one could summarise the more common facilities found on the current generation of analytical electron microscopes by writing AEM = (STEM+TEM+BF+DF) +(EDS) +(EELS) +(SAD+CBD). The reviewer's copy of Williams' book is hard-backed (a soft-backed version is also available) and contains 153 pages of slightly less than A4 size. It is divided into six chapters, three covering the techniques in the first brackets in the above equation and one chapter on the technique in each of the other three brackets. Each of the chapters is a straight reprint of material previously published in *Norelco Reporter* in 1983 and most of the errors in the original still persist.

The author's aim is to convey to both current and potential users both the benefits and limitations of an analytical electron microscope operating at ≤120 kV. The book appears at a time when the "add-ons" that emerged in the 1970s and provided the user with chemical information have become an integral part of the instrument. Although written around the use of Philips equipment, the book does not preclude that from other manufacturers.

It is, by design, a practical book, a reference digest that contains information relative to data collection and reduction. It currently provides the only complete collection of papers that satisfy the above equation. It is lavishly illustrated with photographs and line diagrams—perhaps too much so, as the photographs serve as illustrations rather than the quality of the information that can be obtained. It contains examples of the uses of the equipment that have been published, without being critical of the results obtained. It offers references to most of the standard reference texts on the subjects covered. It could have been more explicit in certain areas, *e.g.*, STEM *vs.* TEM, microprobe *vs.* nanoprobe, determination of displacement vector, which would be beneficial to the inexperienced potential user.

As a reference guide, it should be readily acceptable in an academic and industrial laboratory. Used correctly, it should guarantee a better return on the investment which a new instrument involves. My only reservation is that the author did not update and correct the text.

D. J. Dyson

Plant Drug Analysis. A Thin Layer Chromatography Atlas

H. Wagner, S. Bladt and E. M. Zgainski (translated by Th. A. Scott). Pp. xiv + 320. Springer-Verlag. 1984. Price DM169; $63.10. ISBN 3 540 13195 7; 0 387 13195 7.

This book is an English translation of the German edition entitled "Drogenanalyse. Dünnschichtchromatographische Analyse von Arzneidrogen," published in 1983. It is aimed at all those involved with the analysis of drug substances of plant origin, either for checking purity during drug production or for the identification of unknown materials. As such, the book will be extremely valuable for pharmacists, drug importers and forensic drug analysts. It can be highly recommended.

The mainstay of the book is a collection of 165 high-quality colour photographs of TLC plates illustrating the plant drug separations under discussion in the text. Each plate includes a set of reference compounds alongside the drugs and several methods of detection are often demonstrated on different regions of the chromatogram. The reader is forcibly reminded of the importance of colour in TLC plate evaluation and of the complex spot patterns often encountered with plant drugs. Attempts to tabulate the R_F values and the colours of the principal zones are always less than satisfactory. In particular, the subtle differences in colour observed for different compounds with the same spray reagent are impossible to describe. The authors have over 10 years of experience in recording TLC results using photography and the present book clearly demonstrates the value of this approach.

The book is divided into 15 chapters, each relating to a specific class of plant drugs, *e.g.*, essential oil drugs, alkaloid drugs, coumarin drugs. Within these chapters the material is arranged in sections following a consistent pattern throughout. An initial familiarisation with this layout allows the reader to retrieve rapidly any information required. The extraction of the plant drug in preparation for TLC is first discussed, followed by details of the recommended systems, which are mostly taken from pharmacopoeias. The third section considers detection on the plates after development, making reference to an Appendix where detailed recipes for 39 spray reagents are collected. The fourth section contains an index to the plant drugs for which TLC separations are illustrated, together with the relevant pharmacopoeias and a list of the main chemical constituents in each instance. The final section contains a collection of structural formulae for compounds commonly encountered in the drug class. In each chapter, this introductory material sets the scene for the colour photographs, each accompanied by a comprehensive text identifying the major spots in each chromatogram and providing other appropriate details. At the end of the book, schemes are presented to guide the reader in the identification of unknown plant drugs.

The authors express a hope that the book will prove valuable to those learning the technique of TLC for drug analysis. This is certainly fulfilled by the photographic approach, which would greatly facilitate the analysis of a particular plant drug for the first time. Further, this colourful book should go a long way towards improving the image of TLC by stimulating new users to explore the full potential of the technique and persuading them not to be deceived into underestimating its capabilities by its relative simplicity and low cost. Moreover, for the experienced analyst the book is full of fascinating detail which makes it an invaluable reference for the laboratory.

Richard Gill

Practical Aspects of Gas Chromatography/Mass Spectrometry.

Gordon M. Message. Pp. xvi + 351. Wiley–Interscience. 1984. Price £69.35. ISBN 0 471 06277 4.

This is a book primarily for newcomers to the field. However, it is almost completely devoid of references, which severely limits its value, other than as an introduction to the subject.

The book is divided into four main parts. The first deals with the theory of operation of GC - MS and the second examines the practical aspects of the technique. This is followed by a section on maintenence and fault finding. The final part of the book considers the criteria in selecting a combined GC - MS system. It is to be commended that the author has considered GC - MS to be a single technique, as only by doing this can the synergistic effects of the combination be appreciated. This does, incidently, include the enhanced difficulties associated with fault finding.

Most of the chapters contain useful lists of definitions. The various components in the system are described in detail, including the vacuum system, various forms of ionisation and the signal detectors. GC is similarly treated and there are also

sections on both separators themselves and the transfer lines. Data acquisition and processing is a vital part of GC - MS and this is covered in general terms.

The book contains the minimum of equations necessary to understand the principles involved.

There are several trivial misprints and the use of the singular instead of the plural on a few occasions. There are a few statements that in the reviewer's experience are not true. For example it is stated on p. 74 that high-resolution MS operation has limited use in GC - MS work. There are many papers in the literature using this technique. There are several dubious definitions in the GC section (pp. 103 *et seq.*), including those of dead volume, liquid phase and peak broadening. The author also states that the GC injection port is usually heated significantly above the column temperature (p. 106). He also expects to observe total ion traces of a similar pattern to the GC trace (p. 117). Even with the flame-ionisation detector this is not always the case, let alone with a selective GC detector. Mention is made of the pyroprobe (p. 135), but oddly not in relation to pyrolysis - GC. There is some repetition in the book; for example, comments on carrier gas selection, flow-rates and the arguments in favour of capillary columns.

The chapter on laboratory practice includes topics that are so fundamental to good scientific practice that they have no place in an expensive and specialised book. The same point applies later in the book, in which advice on self-help and the use of service engineers is given. Much of this is common sense. Despite these shortcomings, this is a tidily presented and readable book.

Terry Gough

Analytical Chemistry Progress
Edited by F. L. Boschke. *Topics in Current Chemistry*, 126. Pp. x + 122. Springer-Verlag. 1984. Price DM68; $24.80. ISBN 3 540 13596 0; 0 387 13596 0.

This set of reviews concentrates on the use of lasers in analytical chemistry and aspects of thin-layer chromatography (TLC).

The first article, by R. B. Green, provides an introduction to the use of laser-enhanced ionisation spectrometry (LEI). The laser selectively promotes the analyte of interest to a bound excited state, which is then collisionally ionised in a flame to give a measurable current. The mechanism of the production of ions and the instrumentation required are discussed. The author recognises that so far applications have been limited but claims an increased freedom from interferences and comparable selectivity and sensitivity to conventional flame spectrometry. He ends by proposing that much further work is needed to test discrimination and potential applications.

The second review, by A. Anders, discusses a more conventional use of lasers for the spectrophotometric analysis of biomolecules. A number of selected groups of analytes including chlorophyll, nucleic acids and rhodopsin are discussed, in addition to applications in photomedicine.

In the first of three articles on TLC, D.-G. Volkmann surveys the use of ion-pair chromatography on reversed-phase layers. He introduces the topic with a simplified theory and mechanism and describes applications on both bonded and impregnated layers and the effects of % modifiers, ion-pair reagents and pH. Unfortunately, this review is particularly beset with both spelling errors and unconventional usages (or translations), including "straight" for "normal," and many of the figures are not mentioned in the text. It would clearly have gained from closer editorial attention. The method is only indirectly compared with the widely used HPLC ion-pair system and the only advantage that the author appears to propose for the TLC technique is the ability to use eluents of pH >8, although few examples are offered.

The final well matched pair of papers cover related areas of quantitation in TLC. The first paper, by S. Ebel, comprehensively reviews calibration and evaluation techniques, including their statistical background and the use of external and internal standards, and concludes with a discussion of determination and detection. The final review paper, by I. M. Böhrer, covers the instrumentation required for the evaluation of quantitative TLC and examines the different scanning systems and photometric systems employed and places emphasis on the use of automated equipment. It ends with a useful discussion of the performance (both analytical and economic) of quantitative TLC and argues that it can provide a viable alternative to HPLC.

The value of a compilation of this type to the individual reader is doubtful, as too few analysts are likely to be sufficiently interested in both lasers and TLC to justify purchase. One therefore suspects that this is primarily a volume to be consulted in the library.

Roger M. Smith

ERRATUM

Spectrophotometric Determination of Boron

Jose Aznarez and Jose M. Mir
Analyst, 1985, **110**, 61–64

Page 61, third paragraph of the first column: *for* "8-hydroxy-1-(2-hydroxy-1-naphthylbenzylideneamino)naphthalene-3,6-disulphonic acid (HSNHB) (I)" *read* "3-hydroxy-4-(2-hydroxybenzylideneamino)naphthalene-1-sulphonic acid (HSNHB) (I)."

corrected
Ch. S.
5 Nov 85

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A201 for further information. See page viii.

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