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Any departure from the pure, symmetrical, bias-free input waveform; external d.c. bias; internal d.c. offset, distortion, amplitude or mark-to-space (time) bias; produces a deterioration in the periodic differential electrolytic potentiometric titration curve. The peak potential is decreased, the peak becomes less sharp, the discrimination becomes worse, errors are introduced and the electrodes more quickly become deactivated when any bias or distortion is present in the input current waveform, and this effect is manifest with 2 per cent. contamination of the waveform and destructive at 5 per cent. A d.c. bias causes the peak to split into two peaks. At the same time, the electrodes produce a d.c. output; the symmetrical polarisation shows no d.c. component in the output; and for d.c. offset and amplitude bias, this d.c. output is the same as for classical d.c. differential electrolytic potentiometry. With a time-biassed periodic input of any waveform, a d.c. output of unique properties is produced. This output has the same forms as the classical d.c. differential electrolytic potentiometric curves, but the end-points are sharper, the discrimination is better, the end-points are error-free with dichromate and cerium(IV) titrants, the d.c. potential stabilises very quickly and remains drift-free, even for type II (*b*) curves, the high-quality end-point persists to very low concentrations, the electrodes retain their activity for a long time and the process is independent of frequency. Such titrations can be performed as fast as titrations with visual indicators. The positive errors in classical d.c. differential electrolytic potentiometric titrations with cerium(IV), chromium(VI) and in zero-current potentiometric titrations with vanadium(V) are explained.

**E. BISHOP and T. J. N. WEBBER**

Chemistry Department, University of Exeter, Stocker Road, Exeter, EX4 4QD.

## Monte Carlo Simulation of Matrix Correction Effects

Monte Carlo simulation is useful for the precise evaluation of the effects of complex systems of matrix correction equations (such as occur in spectrographic analysis). If the error distributions for the interfering elements are experimentally determined, that induced by interaction in the correction equation system for the elements subject to interference can be predicted.

**R. J. HOWARTH**

Applied Geochemistry Research Group, Department of Geology, Imperial College of Science and Technology, London, SW7 2BP.

## Spectrophotometric Determination of Low Levels of Mono-, Di- and Triethylene Glycols in Surface Waters

A method is proposed for the determination of mono-, di- and triethylene glycols in surface waters, based on the oxidation of the glycols to aldehydes. These are made to react with 3-methylbenzothiazol-2-one hydrazone hydrochloride to give green cationic chromogens, which are then measured spectrophotometrically at 630 nm. Sample blank values, to compensate for natural interferences, are obtained by omitting the oxidation stage. The method enables glycol levels of upwards of $0 \cdot 5$ mg $l^{-1}$ to be determined, satisfactory recoveries of each glycol being obtained for concentrations of 1 to 5 mg $l^{-1}$, with a precision of 7 per cent., for a range of water samples. A sensitive variation of the method, for the determination of monoethylene glycol alone, is described separately.

**W. H. EVANS and A. DENNIS**

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

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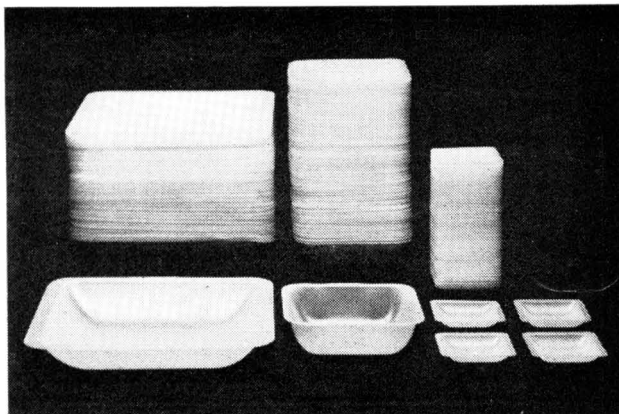
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# Differential Electrolytic Potentiometry with Periodic Polarisation

## Part XXIII.* The Effect of Bias and Distortion on Periodic Differential Electrolytic Potentiometry, the D.C. Output Produced and Time-biassed Differential Electrolytic Potentiometry in Oxidation-Reduction Titrations†

BY E. BISHOP AND T. J. N. WEBBER‡

(*Chemistry Department, University of Exeter, Stocker Road, Exeter, EX4 4QD*)

Any departure from the pure, symmetrical, bias-free input waveform; external d.c. bias; internal d.c. offset, distortion, amplitude or mark-to-space (time) bias; produces a deterioration in the periodic differential electrolytic potentiometric titration curve. The peak potential is decreased, the peak becomes less sharp, the discrimination becomes worse, errors are introduced and the electrodes more quickly become deactivated when any bias or distortion is present in the input current waveform, and this effect is manifest with 2 per cent. contamination of the waveform and destructive at 5 per cent. A d.c. bias causes the peak to split into two peaks. At the same time, the electrodes produce a d.c. output; the symmetrical polarisation shows no d.c. component in the output; and for d.c. offset and amplitude bias, this d.c. output is the same as for classical d.c. differential electrolytic potentiometry. With a time-biassed periodic input of any waveform, a d.c. output of unique properties is produced. This output has the same forms as the classical d.c. differential electrolytic potentiometric curves, but the end-points are sharper, the discrimination is better, the end-points are error-free with dichromate and cerium(IV) titrants, the d.c. potential stabilises very quickly and remains drift-free, even for type II (*b*) curves, the high-quality end-point persists to very low concentrations, the electrodes retain their activity for a long time and the process is independent of frequency. Such titrations can be performed as fast as titrations with visual indicators. The positive errors in classical d.c. differential electrolytic potentiometric titrations with cerium(IV), chromium(VI) and in zero-current potentiometric titrations with vanadium(V) are explained.

No previous work on the use of biassed or asymmetrical waveforms has been discovered in a literature search; earlier work on periodic polarisation has been faulted because no effort seems to have been made to ensure purity and symmetry of waveform, or indeed to examine them, except for one attempt to block d.c. by means of a series capacitor in the generator output,[1] a device that gave very limited success.[2] Both the Heathkit and Advance signal generators that were initially used in this work[2,3] gave unsatisfactory results, which were traced to a d.c. offset in the former and low-frequency distortion in the latter. Addition of a series capacitor did not eliminate all of the d.c. offset, and had no influence on a badly shaped wave, other than to attenuate low frequencies. Moreover, the use of a transformer, even of the constant voltage saturable reactor type, to provide a 50-Hz signal, is productive of waveform distortion and harmonics because of the iron-cored inductance. These observations led to a systematic quantitative examination of the effects of offset and waveform asymmetry on the precision, accuracy and discrimination of end-point location, and on the forms of the titration curves and the speed of electrode response. With a pure periodic waveform, there is no d.c. component in the output from the electrodes, just as there is no periodic component in the output from pure d.c. polarisation. With a biassed or offset waveform, both d.c. and periodic components will be present in the output.

* For details of Part XXII of this series, see reference list, p. 776.
† Presented at the Second SAC Conference, Nottingham, 1968.
‡ Present address: Shell Research Limited, Woodstock Agricultural Research Centre, Sittingbourne, Kent.

Rough predictions of the form of the titration curve for single polarised electrodes *versus* reference and zero-current indicator electrodes, and for pairs of polarised electrodes, were made (Fig. 1 in reference 3) on a basis of the titration analogue model,[4] for fast reactions and 50 per cent. of the particular bias. For example, if there is a d.c. component in the periodic waveform, then, with an oxidant titrant, for a single electrode the end-point will be displaced from the equivalence point by an amount proportional to the magnitude of the d.c. component, and the error will be positive if the d.c. polarisation is cathodic and negative if it is anodic. For paired electrodes, one electrode will be polarised cathodically and the other anodically, and a d.c. differential potential will exist between them. When observed in the sense anode *minus* cathode, there will be a periodic peak superimposed on the d.c. peak. The d.c. output will be the sum of the first and second differentials of the zero-current curve, and the periodic output will broaden, eventually splitting to give two peaks.

## EXPERIMENTAL

Apparatus,[3] solutions and procedures[2] have been described earlier. Various forms of bias were introduced into the pure waveforms, as exemplified in Fig. 1, either as an external or internal d.c. offset, or by waveform shaping to give amplitude or time bias. Amplitude bias and d.c. offset are the same for square waves, but not for sine or triangular waveforms.
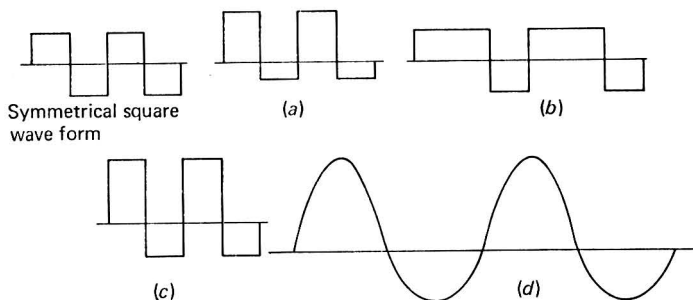


Fig. 1. Biassing of pure waveforms. Initial pure square wave with: (*a*), external d.c. offset; (*b*), a 2:1 mark-to-space (time) bias; (*c*), a 2:1 amplitude bias, which is identical with (*a*) for square wave forms but not for the other waveforms; and (*d*), sine wave with a 2:1 amplitude bias

### D.C. OFFSET—

A limited internal d.c. offset, up to 5 V, could be mixed into the waveform by adjustment of the reference level potentiometer, P9, in the waveform generator,[3] but greater offset could not be produced in this way without introducing distortion into the output signal. Larger offsets were introduced externally by applying a d.c. potential from a battery and potentiometer across the "low" and "earth" connections (normally strapped together) on the waveform generator. After checking the equality of the duration of the half-cycles with the crystal clock, the amount of d.c. present was measured either directly on the oscilloscope, or by increasing the frequency and applying the signal to a d.c. meter.

### AMPLITUDE BIAS—

As is evident from Fig. 1, the amplitude bias is the same as a d.c. offset for square waves, but not for sine and triangular waves. Limitation of facilities at the time prevented further examination of amplitude bias for sine and triangular waveforms.

### TIME BIAS—

Time bias can be obtained by adjustment of the potentiometers P3, P4 and P5 in the generator.[3] For square waves, a perfectly symmetrical, pure signal was first obtained, the half-cycles being identical in amplitude and duration, and the selected amount of bias was introduced. The internal adjustments did not permit more than $\pm20$ per cent. relative variation. The crystal clock was then used to measure the duration of each half-cycle accurately. For sine waves, the clock would not trigger at frequencies less than 14 Hz, and it was necessary to trace the shape of an individual cycle from the oscilloscope screen on

to translucent graph paper, and to cut out and weigh the area representing each half-cycle. This method obviously has poor accuracy; the average error determined by replication was less than 5 per cent. The titration cell was then set up,[3] with the electrode configuration shown in Fig. 3 in reference 2, so that the d.c. and periodic potentials at each of the periodic electrodes could be measured with reference to a standard half-cell or a zero-current electrode, and the periodic and d.c. potential differences between the two periodic electrodes could also be measured. At the same time, d.c. differential electrolytic potentiometry and zero-current potentiometric potentials could also be monitored for comparison.

### RESULTS AND DISCUSSION

EFFECT OF D.C. BIAS ON THE PERIODIC POTENTIAL CURVE—

First, a system showing a modicum of charge-transfer overpotential was examined, the titration of iron(II) with $0.1$ M cerium(IV), with a gradually increasing d.c. offset. The results are shown in Fig. 2, and accord with prediction. These results should be compared with those in Fig. 3 and other figures in reference 2 that show the results with pure, symmetrical, bias-free waveforms.

The presence of a very small (less than 2 per cent.) d.c. offset is sufficient to reduce the sharpness of the periodic potential curve under any given conditions. As the magnitude of the offset increases, the peak gradually broadens, until, at about 30 per cent. offset, it splits into two peaks. The presence of d.c. offset causes deactivation of the electrodes; not only is the relaxation following a concentration perturbation lengthened, but also, after some time, the initially bright and shiny electrodes assume a dull matt surface. With no d.c. offset, potentiometric and periodic end-points agree, but a deviation arises with d.c. offset and increases with increase in d.c. offset. A d.c. offset in the periodic input produces a d.c. component in the periodic output.
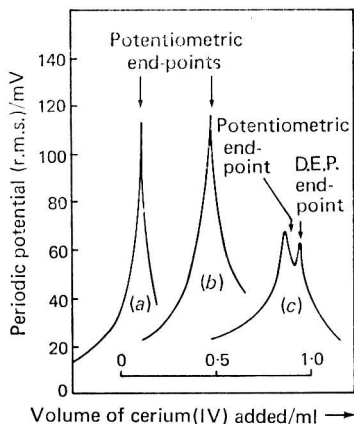


Fig. 2. Variation of the periodic differential electrolytic potentiometric titration curve with increasing d.c. offset in the input signal. Expanded scale end-point region. Titration of 200 ml of $0.0125$ M iron(II) in $0.5$ M sulphuric acid with $0.1$ M cerium(IV). Sine wave, 3 Hz, r.m.s. current density, 25 $\mu$A cm$^{-2}$: (a), 2 per cent. d.c. offset; (b), 5 per cent. d.c. offset; and (c), 40 per cent. d.c. offset

Further titrations were then performed with periodic inputs containing an increasing time bias, and the deterioration in the periodic output was similar to that above with d.c. offset, although instrumental limitations at the time prevented examination of biases above 20 per cent.

Examination of the effect of offset and time bias was extended to reactions of type II (*a*) [titration of iron(II) with dichromate] and type II (*b*) (titration of hydrazine with bromate). The results were again similar to those described above for d.c. offset in the titration of iron(II) and cerium(IV). With increasing bias, the curves became less sharp, the discrimination deteriorated and the periodic end-point deviated increasingly from the zero-current potentiometric end-point.

Finally, the influence of d.c. offset on the electrodically fastest reactions, those of the titration of copper(I) with potassium bromate, was examined. The d.c. differential electrolytic potentiometric peak height is 600 mV at $1 \cdot 0$ $\mu$A cm$^{-2}$, and a solution as near as possible to equivalence was prepared (platinum potential *versus* S.C.E. 520 mV) and a periodic signal of increasing d.c. offset applied to two electrodes; the amplitude of the square wave was maintained constant and the peak to peak output was measured on the oscilloscope. The results are shown in Table I, and indicate that for very fast charge-transfer processes an appreciable d.c. offset at fairly high frequency is necessary before significant reduction in the, probably already clipped, peak potential occurs, although eventually curve splitting into two peaks, as in Fig. 2, occurs.

### TABLE I

VARIATION OF PEAK POTENTIAL FOR AN ELECTRODICALLY FAST SYSTEM
WITH INCREASING D.C. OFFSET

Applied signal: 10-Hz square wave; ballast resistance $5 \times 10^6$ $\Omega$;
current density 10 $\mu$A cm$^{-2}$

| Applied signal/V | | | $\begin{cases} +40 \\ -40 \end{cases}$ | $\begin{matrix} +41 \\ -39 \end{matrix}$ | $\begin{matrix} +42 \\ -38 \end{matrix}$ | $\begin{matrix} +43 \\ -37 \end{matrix}$ | $\begin{matrix} +48 \\ -32 \end{matrix}$ | $\begin{matrix} +50 \\ -30 \end{matrix}$ |
|---|---|---|---|---|---|---|---|---|
| Output signal/mV | .. | .. | 275 | 275 | 275 | 270 | 200 | 170 |

It is therefore abundantly clear that any departure from pure, symmetrical, bias-free periodic waveforms,[2] whether d.c. offset, amplitude or time bias, or distortion will cause a deterioration of the periodic output potential titration curve, and appreciable departures will seriously attenuate the precision and discrimination and introduce errors.

THE D.C. OUTPUT COMPONENT FROM BIASSED OR OFFSET PERIODIC POLARISATION—

By using a d.c. measuring device with a time constant that is long compared with the repetition frequency of the periodic polarising current, any d.c. component can be detected in the output. For an internal or external d.c. offset, the d.c. output produces a differential electrolytic potentiometric curve that is identical with a titration curve produced by classical d.c. differential electrolytic potentiometry at the same current density, but with the differences that in the former instance the electrode response is much faster and the electrodes retain their activity for a longer period. Time biassing, however, produced results of greater benefit and considerable interest.

TIME-BIASSED PERIODIC DIFFERENTIAL ELECTROLYTIC POTENTIOMETRY—

The periodic component of the output from this biassed waveform showed the usual deterioration and error with increasing bias, but the d.c. component proved to have unique and valuable properties. The range of bias available at the time was too small to permit proper investigation of the effect of its magnitude, and so a 5 per cent. bias was selected for examination, and the d.c. current density arbitrarily assigned a value of 5 per cent. of the periodic r.m.s. current density for comparison purposes.

*Titrations at customary concentrations*—With the time-biassed input, examples of each type of oxidation - reduction reaction were examined, and the precision, accuracy and discrimination were compared with those of d.c. and symmetrical periodic differential electrolytic potentiometry. Curves obtained at optimum or near optimum electrical conditions are shown in Fig. 3, on an expanded volume scale in the end-point region, for type I, II (*a*) and II (*b*) reactions.

The titration curves were appreciably sharper, by a factor of about two, than the corresponding d.c. differential electrolytic potentiometric curves of equivalent peak height, with slopes in excess of 50 000 mV ml$^{-1}$, and also sharper than the symmetrical periodic

differential electrolytic potentiometric curves. The response of the electrodes to a concentration perturbation by an increment of titrant in the region of the equivalence point was extremely rapid and considerably faster than for the classical d.c. differential electrolytic potentiometry, by a factor in excess of ten; this result was confirmed by making potential measurements at 5-s intervals. The increase in response speed was most marked with the type II reactions, and is of great benefit. In the iron(II) titration with cerium(IV) after the end-point, although the speed of response remained, potential drift still occurred, which is indicative of changing charge-transfer kinetic parameters; it is possible that gold electrodes could be used with benefit in this titration. The titration curves were independent of the frequency used, over the range from 3 Hz to the upper limit of the generator (1200 Hz), and of the waveform used, whether it was square, sine or triangular. At frequencies below about 20 Hz, the mean periodic potential output traced a curve of the same form as the d.c. potential. However, it was again found that in titrations of iron(II) with cerium(IV) or chromium(VI), the d.c. and periodic peaks failed to coincide. There was again a difference of about 0·01 ml in a 25-ml titration, the pure periodic output peak and the time-biassed d.c. output peak coinciding with the zero-current potentiometric inflection point, and coming before the classical d.c. differential electrolytic potentiometric peak obtained from other electrodes in the same titration. No such differences were found in any of the other titrations examined; all four end-points coincided.

*Titrations at lower concentrations*—Many different titrations were satisfactorily performed with time-biassed differential electrolytic potentiometry at concentrations down to $10^{-5}$ times those just discussed, but the particularly intractable titration of iron(II) with cerium(IV) was chosen for extensive examination, together with classical d.c. differential electrolytic potentiometry and zero-current potentiometry. Titrations with $5 \times 10^{-2}$, $5 \times 10^{-3}$ and $5 \times 10^{-4}$ M cerium(IV) are shown in Fig. 4. The results of these and other titrations are given in Table II for titrant concentrations in the region of $10^{-4}$ M. The assigned current densities at 5 per cent. bias are again 5 per cent. of the r.m.s. periodic current density.
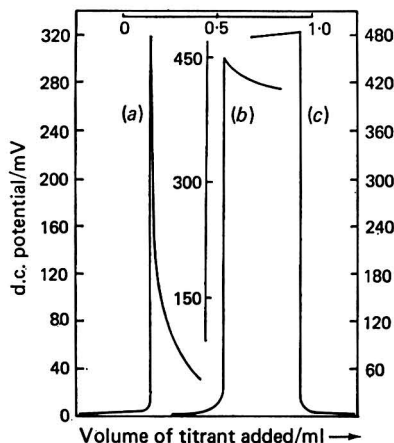


Fig. 3. Titration curve forms obtained by using time bias; end-point region on an expanded volume scale. Sine wave, 100 Hz, 5 per cent. mark-to-space bias, resultant mean d.c. density 1·0 μA cm⁻²: (*a*), titration of 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid with 0·1 M cerium(IV); (*b*), titration of 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid with 0·016 67 M chromium-(VI); and (*c*) titration of 200 ml of 0·003 M hydrazine in 0·1 M potassium bromide and 2·5 M hydrochloric acid with 0·016 67 M potassium bromate
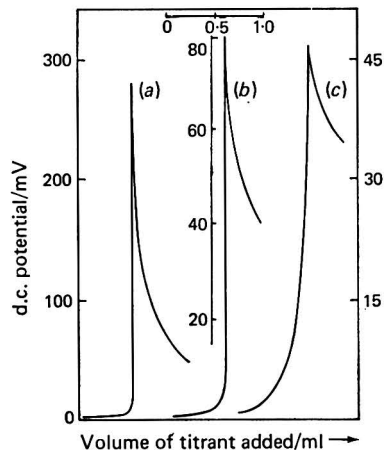
Fig. 4. Time bias d.c. differential electrolytic potentiometric curve forms in the expanded end-point region for lower titrant concentrations. Sine wave, 100 Hz, 5 per cent. mark-to-space bias. Titration of 200 ml of iron(II) in 0·5 M sulphuric acid with cerium(IV) at various concentrations and assigned current densities.

| Curve | $[Fe^{II}]/$ mol l⁻¹ | $(Ce^{IV})/$ mol l⁻¹ | Assigned d.c. density/ μA cm⁻² |
|---|---|---|---|
| (*a*) | $6·25 \times 10^{-3}$ | $5 \times 10^{-2}$ | 1·0 |
| (*b*) | $6·25 \times 10^{-4}$ | $5 \times 10^{-3}$ | 0·25 |
| (*c*) | $6·25 \times 10^{-5}$ | $5 \times 10^{-4}$ | 0·10 |

## TABLE II

TITRATIONS AT LOW TITRANT CONCENTRATION BY TIME-BIASSED
DIFFERENTIAL ELECTROLYTIC POTENTIOMETRY

| Reaction* | Current density/ $\mu A\ cm^{-2}$ | Titre/ml | Standard deviation/ml |
|---|---|---|---|
| (a) | 0·1 | 21·64, 21·59, 21.63, 21·60, 21·58 | 0·02 |
| (b) | 0·1 | 23·11, 23·14, 23·12, 23·17, 23·10, 23·14 | 0·025 |
| (c) | 0·1 | 24·48, 24·53, 24·49, 24·47, 24·48, 24·52 | 0·02 |

* (a)  Titration of 200 ml of a $6·25 \times 10^{-5}$ M solution of iron(II) in 0·5 M sulphuric acid
    with $5 \times 10^{-4}$ M cerium(IV).

   (b)  Titration of 200 ml of a $1·25 \times 10^{-4}$ M solution of iron(II) in 0·5 M sulphuric acid
    with $1·67 \times 10^{-4}$ M chromium(VI).

   (c)  Titration of 200 ml of a $3 \times 10^{-5}$ M solution of hydrazine in 0·1 M bromide *plus*
    2·5 M hydrochloric acid with $1·67 \times 10^{-4}$ M bromate.

Even with titrant concentrations of $5 \times 10^{-4}$ M, the discrimination was still of the same order (about 0·15 per cent.) as the inherent volumetric error, but better than that for d.c. differential electrolytic potentiometry by a factor of about five and much better than that for pure symmetrical periodic polarisation (Fig. 5, reference 2). The response speed of the electrodes, judged by measurement of the time required to reach equilibrium, and the stability of the potentials showed a marked improvement over d.c. differential electrolytic potentiometry at this concentration level. In Fig. 4, it can be seen that there is an increasing tendency towards type II (a) curves as the reactant concentrations decrease. This effect arises from the slowing down of the charge-transfer processes, particularly of cerium, on account of the decrease in the concentration terms in the charge-transfer equation and consequent increase in $\eta_a$.

Results for type II (a) and II (b) reactions are given in Table II; the discrimination was of the same order as for the titration of iron(II) with cerium(IV) and about twice as good as for d.c. differential electrolytic potentiometry. There was also a marked improvement in response speed and stability of the resultant potentials compared with d.c. differential electrolytic potentiometry.

In order to investigate further the prevention of electrode fouling by time-biassed signals, titrations of iron(II) with $5 \times 10^{-4}$ M cerium(IV) solution were performed by the classical d.c. differential electrolytic potentiometric method, and also with a balanced periodic signal of various frequencies of approximately the same r.m.s. current density, 20 $\mu A\ cm^{-2}$, superimposed on the d.c. signal. This produces a synthetic amplitude biassed, but unsymmetrical, signal. There was no appreciable difference in electrode response speed after concentration changes, or any increase in the duration of electrode activity. The effect is similar to, but more deleterious than, that of d.c. offset signals. The special benefits of the time-biassed signal must therefore reside in its particular characteristic of equal anodic and cathodic current excursions of unequal duration.

EXPLANATION OF THE SYSTEMATIC ERRORS IN D.C. DIFFERENTIAL ELECTROLYTIC POTENTIO-
METRY WITH CERTAIN TITRANTS—

The systematic errors reported, but not recognised as such, at an early stage,[5] occur in titrations of iron(II) with cerium(IV), chromium(VI) and vanadium(V), and are real errors as shown by the pipette dilution method[2,3] in which the equivalence point region is traversed in very small increments with diluted titrant. The first peak occurs with symmetrical periodic polarisation and coincides with the d.c. output peak from time-biassed periodic polarisation, both of which coincide exactly with the zero-current potentiometric inflection in cerium(IV) and chromium(VI) titrations, and these are followed after a volume interval of 0·005 to 0·05 ml (depending on the degree of deactivation of the electrodes) by the d.c. differential electrolytic potentiometric peak. In vanadium(V) titrations, the zero-current potentiometric inflection comes first, and d.c., symmetrical periodic, and time-biassed periodic d.c. peaks appear simultaneously after an interval that increases with the speed of the titration, *i.e.*, with decreasing dwell time after the addition of an increment of titrant. Each of the three errors has a different explanation, none of which could be predicted by computer simulation with the program DEP 10.

*Cerium(IV)*—On adding an increment of titrant immediately after the end-point, as indicated by the time-biassed differential electrolytic potentiometric output, the classical d.c. differential electrolytic potentiometric potential, $E_\Delta$, was observed at first to drift downwards, but then drifted back upwards until $E_\Delta$ became greater than it had been before the addition of the tiny increment of cerium(IV). This behaviour continued for several minute increments, equivalent to 0·0005 or 0·005 ml of the original titrant, after equivalence, so making it difficult to locate the end-point precisely. If the latter be taken as the point at which up-and-down drift of $E_\Delta$ after addition of an increment changes to a down-and-up drift, then equivalence and end-points agree, but if the cessation of the drifting is awaited, a positive error of about 0·04 to 0·08 per cent. arises. This error is usually recorded because d.c. differential electrolytic potentiometry, like zero-current potentiometry, is essentially a pseudo-equilibrium technique, whereas the error-free, time-biassed periodic d.c. and the symmetrical periodic potential methods are highly dynamic. The d.c. differential electrolytic potentiometric behaviour is ascribed to a change in the nature of the anode surface when exposed to a minute excess of cerium(IV), leading to a decrease in $k$ and a change in $\alpha$ so that charge-transfer overpotential, $\eta_a$, builds up. This process continues as the cerium(IV) concentration increases, the increase in $\eta_a$ being greater than the decrease in $\eta_c$, until the charge-transfer rate parameters stabilise, and $E_\Delta$ decreases as $\eta_c$ falls, but leaving a residual $\eta_a$ after equivalence. Even with freshly activated electrodes, charge-transfer overpotential is always manifest after the end-point in a cerium(IV) titration, as witness the titration curves for any of the methods. The drifting mentioned does not occur with the time-biassed periodic d.c. output peak, unless the titration is held just past equivalence for many hours; with long waiting periods, the time-biassed periodic d.c. outpeak peak can be persuaded to coincide with the classical d.c. differential electrolytic potentiometric peak. Normally, however, the periodic input current is comparatively large, and so the equilibration of the d.c. potential is very rapid, and the periodicity of the polarisation considerably delays the deactivation of the electrode.

*Chromium(IV)*—With this titrant, the error arises from specific adsorption of chromium(VI) on the anode surface.[6] This adsorption has been shown to be concentration-dependent and to occur at low dichromate concentrations. The adsorption therefore increases as the concentration of excess dichromate builds up after equivalence to the point of complete electrode coverage, and this point corresponds to the false, late d.c. differential electrolytic potentiometric end-point. The specific adsorption is encouraged by electrosorption on the anode, and the conditions at the anode surface represent a progressively more oxidising situation than mass transfer from the bulk of the solution would predict. Again, the accelerated potential equilibration of symmetrical periodic and time-biassed periodic polarisation are antagonistic to the slow adsorption process, and the large r.m.s. current densities and the periodicity of the polarisation minimise the adsorption and its effect.

*Vanadium(V)*—In this instance, classical d.c., symmetrical periodic, and time-biassed periodic d.c. output differential electrolytic potentiometry all give the correct answer; it is the zero-current potentiometric inflection that is wrong. As an electrode kinetic study of the vanadium(V) - vanadium(IV) system has shown,[6] the charge-transfer process is very slow; the exchange current is very small, and so relaxation of the zero-current potential after a concentration perturbation by the addition of an increment of titrant is very slow. It has been reported[6] that the zero-current potential, even in heavily poised solutions equimolar in the two oxidation states, takes an excessive time to reach equilibrium. If, as is commonly the practice with titrimetric reactions of reasonable $Q$ values, "equilibrium" is interpreted as a drift of less than $1 \, mV \, min^{-1}$, then the pseudo-equilibrium potential will lag behind the true equilibrium potential by a significant amount, especially in the equivalence point region where concentrations are low. The zero-current potentiometric curve therefore rises (or falls) prematurely and the inflection is early. That this fact has not previously been recorded is surprising; zero-current potentiometry has been accepted as the reference method in the titrimetric determination of vanadium for many decades. It can only be concluded that sufficiently pure vanadium and iron compounds have never previously been matched to reveal this, admittedly small, systematic error. It has also been shown that vanadium species, predominantly vanadium(V), are adsorbed on electrodes,[6] and this adsorption aggravates the deviation explained above. That twin polarised electrodes give the correct answer arises from the enhanced rate of potential equilibration under the

passage of current, and periodic polarisation further minimises adsorption and encourages the retention of electrode activity.

## CONCLUSIONS

No previous examination of the various types of d.c. bias or of the form and accuracy of the titration curve has been reported, nor, with one exception,[1] has any effort been made to detect or eliminate d.c. bias. Any form of bias or distortion causes a deterioration in the periodic titration curve as well as introducing errors of end-point location, and, with d.c. offsets, marked electrode deactivation. For optimum results, the periodic waveform must be accurately shaped so as to remove all trace of offset, bias and distortion. There is then no d.c. component in the periodic output, but the presence of even traces of such deviations produces a d.c. output as well as a periodic output.

The most important result of this investigation is the discovery of the unique properties of time-biassing of the periodic signal. Such an input produces a d.c. differential titration curve that shows an improvement in precision and discrimination over both classical d.c. differential electrolytic potentiometry and symmetrical periodic differential electrolytic potentiometry. It retains the advantages of periodic differential electrolytic potentiometry in a great improvement in electrode response speed, stability of the electrode potentials and minimisation of electrode fouling and deactivation. The alternating anodisation and cathodisation of each electrode keeps it active, while the large over-all impressed current density accelerates the electrode response. Both of these factors are important, particularly in dilute solutions, in automatic operation and process control. Also, as with symmetrical periodic polarisation, the end-point is brought into agreement with the equivalence point.

Errors that arise in d.c. differential electrolytic potentiometry with cerium(IV) and chromium(VI) titrations, and in zero-current potentiometry with vanadium(V) titrations, have been satisfactorily resolved from electrode kinetic studies.

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   NOTE—References 2, 3, 4 and 5 are to Parts XXII, XXI, XVII and II, respectively, of this series.

# Monte Carlo Simulation of Matrix Correction Effects

By R. J. HOWARTH

(*Applied Geochemistry Research Group, Department of Geology, Imperial College of Science and Technology, London, SW7 2BP*)

Monte Carlo simulation is useful for the precise evaluation of the effects of complex systems of matrix correction equations (such as occur in spectrographic analysis). If the error distributions for the interfering elements are experimentally determined, that induced by interaction in the correction equation system for the elements subject to interference can be predicted.

In spectrographic systems the observed concentrations of both major and trace elements may have been affected by errors that are dependent on the concentrations of other elements present in the sample. The magnitude of these errors, and the form of the functional relationship between the affected element and the interfering elements, will vary with the nature of the sample, the system of correction equations being generally referred to as "matrix corrections." This functional relationship can be represented in the general form

$$C_j^{\cdot} = f_j (C_j, x_1, x_2, \ldots, x_1, \ldots, x_n) \qquad \cdots \qquad \cdots \qquad \cdots \qquad (1)$$

where $C_j^{\cdot}$ is the corrected concentration of the $j$th affected element; $C_j$ the observed concentration of the $j$th affected element; $x_1$ the observed concentration of the $i$th interfering element $(i = 1, \ldots, n)$; and $f_j (\ )$ the generalised functional relationship for the $j$th affected element.

The form of $f_j (\ )$ is generally determined by observations made on known samples spiked with various concentrations of the interfering elements. Any element that has been corrected may, in its turn, be used in further correction equations, so building up a complex system for the simultaneous correction of all the elements being determined. However, direct appraisal of the effects of such equation systems cannot be carried out in more than qualitative terms.

The detailed matrix corrections will normally be specific to particular laboratories as they will depend on the instrumentation, the calibration techniques used and the nature of the samples being analysed. The general principles of emission-spectrographic and X-ray fluorescence analysis have been outlined by Ahrens and Taylor[1] and Norrish and Chappell,[2] respectively, and matrix correction equations have been used in both emission-spectrographic and, to a lesser extent, X-ray fluorescence analysis. The papers of Tennant and Sewell[3] and Leake *et al.*[4] may be cited as examples of typical laboratory applications.

The purpose of this paper, however, is to indicate how Monte Carlo simulation can be used as a tool to assess quantitatively the effects of error propagation through a battery of matrix correction equations, an aspect that has not previously been investigated. It will be illustrated with examples drawn from an emission-spectrographic system, although the technique could be applied to any situation where complex inter-element interference occurs.

## Monte Carlo Simulation—

A Monte Carlo simulation is based upon the development of a mathematical model (the matrix correction equation system) that accurately represents the real-world situation to be investigated. The concepts of Monte Carlo simulation are discussed in a number of texts.[5–7] The sources of random error are represented in the model by pseudo-random number generators, random values being drawn from populations that have the same probability distribution and parameters (in this instance the mean and standard deviation) as those in the real-world system. The model is translated into a computer program and simulation runs are conducted by the computer to represent randomly selected real-world trials.

A suitable basis for the simulation is the Muller method[8] of generating pairs of random uncorrelated values ($A$ and $B$) from a Gaussian parent population of mean $m$ and standard deviation $s$, these values having been determined by observation of the real-world system it is desired to simulate. Let $U$ and $V$ be independent random variables uniformly distributed

in the interval $(0, 1)$; then, the probability distribution for $U$ and $V$ is described by

$$P(Y \leqslant y) = \begin{cases} 0 \text{ if } y < 0 \\ y \text{ if } 0 \leqslant y \leqslant 1 \\ 0 \text{ if } y > 1 \end{cases}$$

The majority of computer centres have library programs for generating uniform random variables of this type. Now

$$A = m + s \sqrt{-2 \ln U} \cos (2\pi V) \qquad \dots \qquad \dots \qquad \dots \qquad (2)$$

$$B = m + s \sqrt{-2 \ln U} \sin (2\pi V) \qquad \dots \qquad \dots \qquad \dots \qquad (3)$$

Because the Monte Carlo simulation involves random values, the results obtained are subject to statistical fluctuations; thus, the larger the number of trials carried out, the more precise will be the final answer.

SIMULATION OF AN EMISSION-SPECTROGRAPHIC SYSTEM—

As an example of the application of this method to a real system, we will briefly consider simulation of the matrix correction equation system for the ARL 29000B direct-reading optical spectrograph that is being used by the Applied Geochemistry Research Group for rapid, low-precision analysis of approximately 50 000 stream sediment samples in order to compile a regional geochemical atlas of England and Wales.

With such an instrument we have the following defects: spectral interference between the lines present for two (or more) elements; a background effect, principally caused by continuous radiation, scattering, or fine spectra due to molecular emission; and thirdly, the arc effect, which is an intensification or diminution of the intensity of a given spectral line caused largely by variation in the temperature of the arc as a result of differing rock matrix composition.[1] It is necessary to evaluate the effect that variations in the major element determinations have upon the trace-element values, by acting through the system of matrix correction equations.

Analytical control is based on a series of eight representative natural standards (stream sediment samples from streams draining known homogeneous rocks) and two synthetic standards spiked with either a low or a high trace-element concentration. From the initial period of operation of the spectrograph it was possible to obtain the mean and standard deviation values for the element concentrations in all of the standards, based on a large number of replicate determinations for the major elements (aluminium, calcium, iron, potassium, magnesium and silicon) and each of twenty-four trace elements. If we assume, on the basis of the observed behaviour of the element, that the error distribution for the major elements is Gaussian and that for the $i$th element it is distributed with mean $m_1$ and standard deviation $s_1$, then we can simulate the major element variation for any matrix type by substituting the appropriate values of $m_1$ and $s_1$ into equations of the form of equations (2) and (3). The observed uncorrected mean trace-element values [$C_j$ of equation (1)] should also be recorded.

For each standard, 1000 simulated sets of major element values were drawn at random by using the Muller method, and the corresponding matrix-corrected trace-element values [$C_j^{\cdot}$ of equation (1)] were evaluated. The change, or perturbation, of the initial values caused by the correction equations was then calculated as a percentage ratio. For the $j$th trace element the perturbation is given by

$$pu_j = 100 \ (C_j^{\cdot} - C_j)/C_j \text{ per cent.} \qquad \dots \qquad \dots \qquad \dots \qquad (4)$$

These data were output by the computer program in the form of histograms, together with the mean, standard deviation and maximum and minimum perturbation values for each trace element for each of the ten standards.

As an additional check on the validity of the method, the total percentage of oxide was calculated for each set of simulated major element values. These results had means acceptably close to 100 per cent. for all standards.

SIMULATION RESULTS OBTAINED—

It is to be expected that any correction equation of the form

$$C_j^{\cdot} = a_0 C_j + a_1 x_1 + a_2 x_2 + \dots + a_n x_n \qquad \dots \qquad \dots \qquad \dots \qquad (5)$$
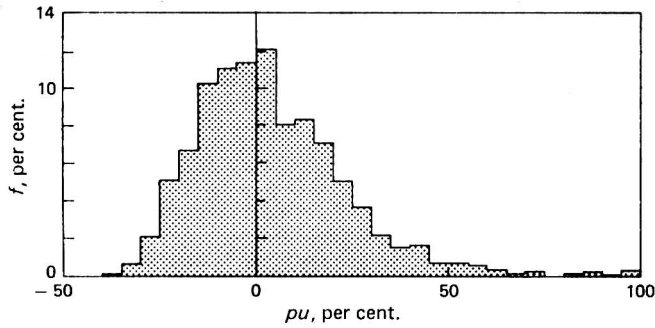
Fig. 1. Histogram of percentage frequency ($f$) of perturbation values ($pu$, per cent.) for strontium in the limestone-derived stream sediment control sample

(where the $a_i$ are coefficients) will yield a Gaussian distribution for $\overset{\bullet}{C_j}$.[9] That the frequency distributions for the perturbation values were Gaussian in all instances where the nature of the correction equations would indicate it, was confirmed by testing against fitted Gaussian distributions by using the Kolmogorov - Smirnov statistic.[10]

This reproductive property of the normal distribution does not apply to non-linear correction equations. For example, Fig. 1 shows the positively skewed perturbation frequency distri-
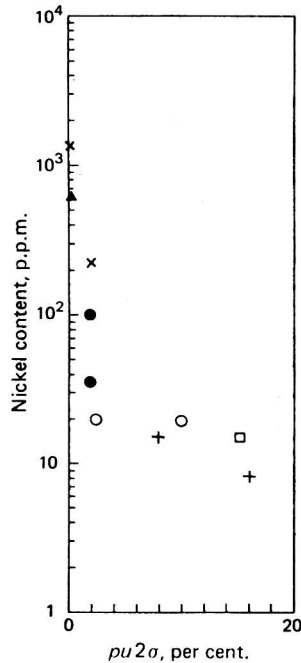


Fig. 2. Variation of percentage perturbation at two standard deviations ($pu$ $2\sigma$, per cent..) with increasing average nickel content in the control samples. Lithologies of the stream sediment source rocks are shown: shale ($\bullet$); sandstone ($\bigcirc$); limestone ($\square$); granite ($+$); basic igneous ($\blacktriangle$); and synthetic standard ($\times$)

bution for strontium in the limestone standard, resulting from a correction equation of the form

$$Sr^* = (Sr + a_1Ca)/(1 + a_2Al + a_3Ca + a_4Fe + a_5Si)$$

The general reduction in the size of the relative perturbation with increase in the trace-element content is typified by the behaviour of nickel (Fig. 2). This element is corrected solely for calcium and therefore shows a Gaussian distribution for the perturbation values. The graph shows the relative perturbation at two standard deviations and indicates that we can expect that only 5 per cent. of the nickel determinations would be perturbed by more than the values shown.

While it has to be remembered that each analytical system will yield a unique matrix correction system (and hence perturbation effects), it is of interest to note how the behaviour predicted by simulation compared in this instance with the results obtained in practice.

Table I shows that, for the majority of elements, the mean perturbation value for the synthetic standard spiked with low element concentrations is close to zero (per cent.). However, there are considerable variations in the magnitudes of the perturbation standard deviation. The spread of values obtained in a typical set of replicate analyses (made on one day, very much later in the project than the data used for the simulation) is indicated by the precision, defined here as twice the element standard deviation divided by the mean, in terms of concentration. It is clear from Table I that for most elements the effect of the matrix corrections has been to increase the precision value to some extent, and that when this increase has been in excess of a factor of 1·3 compared with the uncorrected value (for silver, arsenic, beryllium, cobalt, lithium, molybdenum, phosphorus, tungsten and zirconium) it correlates very well with high-perturbation standard deviations obtained by the earlier simulation of the system. Some difficulty would therefore be expected in obtaining reliable low-level analyses for these elements. It was found necessary in practice to use alternative methods of analysis for arsenic, molybdenum, cadmium and zinc; silver, phosphorus, tungsten and zirconium have not been used in the preparation of geochemical maps. The simulation results have therefore been well borne out in day-to-day experience of the rapid, low-precision analytical system necessary to cope with the very large number of multi-element analyses necessary for a geochemical reconnaissance of this type.

TABLE I

COMPARISON OF SIMULATED AND ACTUAL PERTURBATION EFFECTS FOR
A SYNTHETIC STANDARD

| Element ($j$) | | | | $\bar{c}_j^*$ | $\bar{p}u_j$ | $\sigma_{pu}$‡ | $\phi_c/\phi_{uc}$§ |
|---|---|---|---|---|---|---|---|
| Ag | .. | .. | .. | 3 | 3 | 92 | 3·32 |
| As | .. | .. | .. | 719 | 0 | 86 | 1·87 |
| Ba | .. | .. | .. | 146 | 0 | 6 | 1·06 |
| Be | .. | .. | .. | 26 | −1 | 17 | 1·44 |
| Bi | .. | .. | .. | 34 | 0 | 1 | 1·00 |
| Cd | .. | .. | .. | 26 | −1 | 28 | 1·03 |
| Co | .. | .. | .. | 16 | 0 | 62 | 2·23 |
| Cr | .. | .. | .. | 190 | 0 | 3 | 1·10 |
| Cu | .. | .. | .. | 75 | 0 | 1 | 1·00 |
| Ga | .. | .. | .. | 18 | 0 | 0 | 1·00 |
| Li | .. | .. | .. | 47 | 0 | 20 | 1·81 |
| Mn | .. | .. | .. | 60 | 0 | 4 | 1·03 |
| Mo | .. | .. | .. | 6 | −2 | 49 | 1·69 |
| Ni | .. | .. | .. | 166 | 0 | 1 | 1·03 |
| P | .. | .. | .. | 496 | 2 | 69 | 1·54 |
| Sc | .. | .. | .. | 21 | 0 | 12 | 1·00 |
| Sn | .. | .. | .. | 252 | 0 | 1 | 1·07 |
| Sr | .. | .. | .. | 197 | 1 | 13 | 1·02 |
| Ti | .. | .. | .. | 312 | 0 | 2 | 1·06 |
| V | .. | .. | .. | 240 | 1 | 6 | 1·21 |
| W | .. | .. | .. | 154 | −1 | 19 | 1·59 |
| Zn | .. | .. | .. | 27 | 2 | 46 | 1·16 |
| Zr | .. | .. | .. | 200 | 2 | 84 | 2·50 |

\* Mean concentration (p.p.m.) of matrix corrected results (11 samples).
† Mean simulated perturbation (per cent.) for matrix correction (1000 trials).
‡ Standard deviation of perturbation values (per cent.) for matrix correction (1000 trials).
§ Ratio of precision [$\phi = 2$ (standard deviation/mean percentage)] for corrected ($\phi_c$) and uncorrected ($\phi_{uc}$) results (11 samples).

## CONCLUSION

Visual evaluation of the effects of multi-component correction equations cannot be carried out in more than qualitative terms. However, Monte Carlo simulation allows the over-all magnitude of the matrix corrections to be evaluated precisely, and affords a method of comparison between the various corrected elements that helps to assess the relative magnitude of the perturbation effects. The method is easily applied to the most complex of matrix correction equation systems. It is economical in computer time and methods are available for determining the optimum number of trials for the evaluation of a given model.[11]

It is not intended that the specific results reported here should be applied to other emission-spectrographic systems, but rather encourage the investigation of other analytical system interactions by using simulation techniques when it is of interest to separate the effects of errors in the determination of the uncorrected element values from changes induced through the application of multi-component correction equations.

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ห้องสมุด กรมวิทยาศาสตร์

# Spectrophotometric Determination of Low Levels of Mono-, Di- and Triethylene Glycols in Surface Waters

By W. H. EVANS and A. DENNIS

(*Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE*1 9*NQ*)

A method is proposed for the determination of mono-, di- and triethylene glycols in surface waters, based on the oxidation of the glycols to aldehydes. These are made to react with 3-methylbenzothiazol-2-one hydrazone hydrochloride to give green cationic chromogens, which are then measured spectrophotometrically at 630 nm. Sample blank values, to compensate for natural interferences, are obtained by omitting the oxidation stage. The method enables glycol levels of upwards of 0·5 mg l$^{-1}$ to be determined, satisfactory recoveries of each glycol being obtained for concentrations of 1 to 5 mg l$^{-1}$, with a precision of 7 per cent., for a range of water samples. A sensitive variation of the method, for the determination of monoethylene glycol alone, is described separately.

Mono-, di- and triethylene glycols, frequently mixed with volatile aliphatic alcohols, have been used as de-icing agents for aircraft and airfield runways. Airfield drainage may subsequently contaminate surface waters with low levels of these glycols. It has been recommended that mono- and diethylene glycol concentrations should not exceed 1 mg l$^{-1}$ in water reservoirs,[1] but no methods for measurement at these levels appear in the literature. Because of the polarity of the glycols it is not possible to concentrate them quantitatively by solvent extraction, therefore their determination must be accomplished in the aqueous phase. This factor, associated with low sensitivity, excludes many instrumental techniques and also thin-layer and gas - liquid chromatography. For similar reasons, spectrophotometric methods involving the specific reaction of glycols with 1-naphthol in sulphuric acid,[2] or methods involving general reactions for aliphatic hydroxyl groups, such as dichromate oxidation,[3,4] the formation of vanadium(V) hydroxyquinolinates[5] or reaction with ammonium cerium(IV) nitrate,[6,7] are unsuitable.

Accordingly, the conversion of the glycols into other compounds has to be considered. This conversion could be achieved by oxidation of the primary alcohol groups to aldehyde or carboxyl groups, but methods for the determination of these functional groups are, in general, equally insensitive. The determination of aldehydes has been reviewed by Altshuller, Cohen, Meyer and Wartburg.[8] The formation of Schiff's bases[9] or 2,4-dinitrophenylhydrazones[10] is not specific to aldehydes and methods based on their formation have low sensitivity, while the reaction with phenylhydrazine and potassium hexacyanoferrate(III)[11] requires the presence of a minimal amount of water. Cleavage of the vicinal glycol group to formaldehyde,[12] followed by reaction with 1,8-dihydroxynaphthalene-3,6-disulphonic acid (chromotropic acid),[13] would enable only one of the glycols to be determined down to the level of 1 mg l$^{-1}$. Three reagents that give cationic chromogens with aldehydes are 2-hydrazinobenzothiazole,[14] 2-hydrazinobenzothiazole with *p*-nitrobenzenediazonium tetrafluoroborate[15] and 3-methylbenzothiazol-2-one hydrazone hydrochloride (MBTH).[16] The first two of these reagents require alkaline conditions and the third requires acidic conditions. The reagents have shown superior sensitivity to aldehydes when compared with chromotropic acid and related compounds.[17] 2-Hydrazinobenzothiazole and MBTH react only with aliphatic aldehydes, and the latter is more sensitive to a wider range of these compounds. More recently, the sensitivity of MBTH has been greatly improved,[18] and the reaction has been applied to the determination of olefins after oxidation,[19] to compounds containing 2-aminoethanol and ethylenediamine fragments[20] and to sugars containing aldehyde groups or polyhydroxyaldehyde precursors.[21]

The sensitivity of MBTH to a wide range of aliphatic aldehydes suggested that, provided suitable conditions for the oxidation of the glycols to aldehydes can be achieved, this reagent

might be the basis for a sensitive method for the determination of glycols in surface waters. A method is proposed, based on the investigations described, whereby the three glycols can be monitored in surface waters at concentrations of upwards of $0.5$ mg $l^{-1}$.

## METHOD

REAGENTS—

These should be of analytical-reagent grade when available; solutions can be prepared with de-ionised water.

*Sulphuric acid*, 4 N.

*Potassium permanganate solution*, 0·0126 M—Dissolve $0.2$ g of potassium permanganate in water and dilute the solution to 100 ml.

*Sodium arsenite solution*, 0·07 M—Dissolve $9.1$ g of sodium arsenite in 100 ml of water. For use, dilute 10 ml of this solution to 100 ml.

*3-Methylbenzothiazol-2-one hydrazone hydrochloride (MBTH)*, 2·0 *per cent*. m/V *solution*— Dissolve 2 g of reagent in 100 ml of water.

*Iron(III) chloride - sulphamic acid solution*—Dissolve 2 g of iron(III) chloride hexahydrate and 3 g of sulphamic acid in water and dilute the mixture to 100 ml.

*Standard glycol solutions*—Mix 10 g of each glycol with water in separate 1-litre calibrated flasks, and dilute 50 ml of each solution to 1 litre to give solutions containing 500 mg $l^{-1}$ of glycol. Immediately prior to use, dilute 5 ml of each of these standards to 500 ml to give standard solutions containing 5 mg $l^{-1}$ of glycol.

PROCEDURE—

Measure 5 ml of sample (after allowing it to settle or filtering it through pre-washed cotton-wool), or a suitable aliquot diluted to 5 ml, into a 10-ml calibrated flask, and also prepare a reagent blank with 5 ml of de-ionised water. To each flask add 0·5 ml of sulphuric acid followed by 1 ml of potassium permanganate solution, mix well, and immerse the flasks in a boiling water bath for exactly 5 minutes. Withdraw them from the water-bath and remove any unreacted permanganate with 1 ml of sodium arsenite solution. Add 1 ml of MBTH reagent solution and immerse the flasks in the water-bath for a further 6 minutes. Remove them, cool to room temperature, transfer 1 ml of iron(III) chloride - sulphamic acid reagent by pipette into each flask and dilute to 10 ml in each instance. Allow to stand for 20 minutes and read the optical density of each solution at 630 nm in a clean 2-cm cell with water in the reference cell. The net optical density of the sample is obtained by subtracting the reagent blank value.

To compensate for natural interferences in surface waters, a sample blank value should be obtained with the permanganate oxidation stage omitted. Measure 5 ml of filtered or settled sample, or a suitable aliquot diluted to 5 ml, into a 10-ml calibrated flask; also prepare a reagent blank with 5 ml of de-ionised water. Add in order 0·5 ml of sulphuric acid, 1 ml of sodium arsenite solution and 1 ml of MBTH reagent solution, mixing after each addition, and immerse the mixture for 6 minutes in a boiling water bath. Ascertain the optical density of the sample blank, less that due to the reagent blank, by the procedure described previously after the addition of 1 ml of iron(III) chloride - sulphamic acid reagent.

The optical density due to glycols, obtained by subtracting this natural blank value from the net sample optical density, can be expressed as the concentration of the glycol, if known, or in terms of a specific glycol from the calibration graphs.

CALIBRATION—

To a series of 10-ml calibrated flasks add, with a pipette, 0, 1, 2, 3, 4 and 5 ml of the standard solution containing 5 mg $l^{-1}$ of glycol, and dilute to 5 ml with de-ionised water when necessary. Ascertain the optical densities, less that due to the reagent blank, by the procedure described. The resulting calibration graphs of optical density against concentration of glycol are linear in the range from 0 to 5 mg $l^{-1}$ for each glycol.

## DISCUSSION

The oxidants used most frequently in organic chemistry include permanganate, chromic acid or systems involving metal ions such as cerium(IV), manganese(III), cobalt(III) or vana-

dium(IV). The magnitudes of the electrode potentials of various oxidising media indicate that cerium(IV) in perchloric acid ($E°$, 1·71 V) or nitric acid ($E°$, 1·61 V) and acidified permanganate ($E°$, 1·52 V) are more effective for oxidation than acidified dichromate ($E°$, 1·33 V) and other systems. These figures may not reflect, however, the effectiveness of their oxidation properties when applied to organic substrates.

Chromium(VI) has been extensively used to oxidise primary alcohols but invariably further oxidation to carboxylic acids, or side-reactions yielding esters via the hemiacetals, occur. Non-substituted 1,2-diols, such as monoethylene glycol, are normally oxidised to the dialdehyde or dicarboxylic acid without cleavage.[22] Cerium(IV), in an acidic medium, oxidises alcohols via complex intermediates that may not easily be oxidised further, while 1,2-diols may be cleaved[22] and would yield formaldehyde from monoethylene glycol with a resulting disproportionately high molar extinction[16] compared with the extinctions obtained with di- and triethylene glycols. It has been suggested that alkaline conditions are more effective than neutral or acidic conditions for the permanganate oxidation of primary alcohols, while 1,2-diols are not noticeably cleaved to aldehydes with permanganate.[22]

An oxidising system, involving the use of acidic or neutral conditions, was required that would convert the hydroxyl groups of mono-, di- and triethylene glycol into aldehyde groups, while, at the same time, minimising further oxidation to the corresponding carboxylic acid compounds. Of several oxidising systems initially investigated, dichromate in dilute acetic or sulphuric acid and neutral permanganate produced no measurable aldehyde at temperatures up to 100 °C; permanganate in dilute sulphuric acid gave a good response for aldehydes for oxidation at 100 °C. One of the factors normally determined in water examination is the 4-hour permanganate value, measured in acidic conditions. For river waters this value has been related empirically to the permanganate consumed on heating the sample for 30 minutes at 100 °C, the consumption being doubled for the latter conditions.[23] The retention of acidified permanganate as oxidant would therefore be an advantage for this method, an allowance for the normal permanganate consumption of river waters being incorporated in the amount of permanganate used.

The total determination involved a number of stages that required careful investigation in order to obtain a reproducible response. For the oxidation stages, the concentration of permanganate, time of reaction required for oxidation, acidity and concentration of reductant for removal of excess of permanganate required particular attention. For the spectrophotometric stages the factors that required investigation were the concentration of MBTH reagent, the time of reaction to form the intermediate azine, the acidity and, finally, the conditions for oxidation, with iron(III) chloride, of the reagent MBTH to a reactive cation and subsequent formation of the final blue or green cationic chromogen.

### Spectrophotometric determination with 3-methylbenzothiazol-2-one hydrazone hydrochloride (MBTH)

An interfering opalescence in the colour development of the MBTH procedure was originally controlled by dilution with acetone, the resulting method losing sensitivity.[16] Subsequently, Hauser and Cummins[18] substituted sulphamic acid for acetone for controlling this opalescence and achieved a six-fold increase in sensitivity for formaldehyde, the final reaction volume being reduced to 12 ml. Preliminary work indicated that, as the reagent concentration decreased with increasing volume of solution, so the relative response to the aldehyde precursors decreased; this effect was only partially overcome by increasing the concentration of MBTH. To obtain the necessary sensitivity the final solution volume was therefore limited to 10 ml. Because the addition of several reagents involved up to 5 ml of solution, sample volumes used in this determination could not exceed 5 ml.

Each glycol oxidation product on reaction with MBTH produced a green chromogen that gave an absorbance maximum in the wavelength region of 630 nm. The effects of variations in the reaction conditions are exemplified by the net optical densities in Table I. Values in italic type are the averages (per mg l$^{-1}$) of eight series of readings obtained for 1, 2, 3, 4, 5 and 6 mg l$^{-1}$ of each glycol by a single operator who used the procedure described under Method. The average coefficient of variation from the average response at each level for each glycol for this series of readings was 3 per cent. In no instance did the averages of other operators differ from the average values by more than $\pm 3$ per cent. The sensitivity

of each aldehyde precursor to MBTH concentration reached a maximum with 1 ml of 1·5 per cent. of reagent, theoretically a many-fold excess, and declined as the concentration increased further. One millilitre of 2 per cent. MBTH was chosen as being the optimum concentration of reagent, thereby avoiding excessively high optical densities with the higher concentrations of monoethylene glycol, which might prove difficult to read accurately on some spectrophotometers.

The extent of the reaction at 100 °C did not alter over the time span 2 to 10 minutes, although response to the product of oxidation of diethylene glycol declined slightly for a reaction time of 10 minutes; a reaction time with MBTH of 6 minutes was therefore selected.

Oxidation of the MBTH reagent to a reactive cation and subsequent formation of the green cationic chromogen was achieved with iron(III) chloride, the opalescence being controlled by sulphamic acid. The response to each aldehyde precursor increased as the volume of 2 per cent. iron(III) chloride - 3 per cent. sulphamic acid reagent increased. A volume in excess of 1 ml of this reagent produced increases in the reagent blank that were disproportionate to the increase in response obtained for both di- and triethylene glycols, and hence 1 ml of the composite reagent was used in this method. It was necessary to cool the reaction mixture to room temperature prior to this addition. The final colour formed reached a maximum response after 15 minutes and remained stable for a further 15 minutes. Measurement 20 minutes after addition of the reagent was therefore recommended.

CONDITIONS FOR OXIDATION OF GLYCOLS TO ALDEHYDES—

The effects of varying the reagent conditions for oxidation are summarised in Table II by the net optical densities shown; values in italic type are averages, as in Table I. A constant response was obtained for mono- and triethylene glycols, at the levels indicated, with 1 to 2 mg

TABLE I

EFFECT OF VARYING REAGENT CONDITIONS ON SPECTROPHOTOMETRIC DETERMINATION WITH MBTH

| | Optical density ($\times 1000$)* | | | | | |
| | Monoethylene glycol/mg l$^{-1}$ | | Diethylene glycol/mg l$^{-1}$ | | Triethylene glycol/mg l$^{-1}$ | |
| Variable | 2 | 4 | 2 | 4 | 2 | 4 |
|---|---|---|---|---|---|---|
| MBTH concentration, † per cent. | | | | | | |
| 0·5 | 220 | 436 | 60 | 138 | 91 | 198 |
| 1·0 | 292 | 580 | 90 | 190 | 115 | 242 |
| 1·5 | 308 | 620 | 104 | 212 | 127 | 259 |
| 2·0 | *260* | *520* | *106* | *212* | *118* | *236* |
| 3·0 | 186 | 374 | 93 | 173 | 98 | 193 |
| Time of reaction‡/ minutes | | | | | | |
| 2 | 261 | 528 | 102 | 200 | 121 | 246 |
| 4 | 257 | 537 | 97 | 212 | 118 | 247 |
| 6 | *260* | *520* | *106* | *212* | *118* | *236* |
| 8 | 252 | 524 | 92 | 208 | 117 | 234 |
| 10 | 256 | 528 | 96 | 196 | 118 | 240 |
| Volume of reagent added§/ml | | | | | | |
| 0·5 | 142 | 277 | 51 | 115 | 75 | 138 |
| 0·75 | 206 | 442 | 72 | 166 | 98 | 200 |
| 1·0 | *260* | *520* | *106* | *212* | *118* | *236* |
| 1·25 | 274 | 600 | 116 | 234 | 130 | 268 |
| 1·50 | 325 | 670 | 112 | 242 | 135 | 274 |

\* Values in italic type are averages.
† Concentration in 1 ml of reagent.
‡ Reaction at 100 °C with 1 ml of 2·0 per cent. MBTH.
§ 2 per cent. iron(III) chloride - 3 per cent. sulphamic acid reagent.

of permanganate, while a constant level was obtained for diethylene glycol for the range 1·5 to 2 mg of permanganate. The maximum permissible sample volume employed in this procedure is 5 ml and it can be calculated that a water sample with a 4-hour permanganate value of 10, representing a badly contaminated river water, would consume 200 $\mu$g of permanganate. If the empirical relationship of a doubled consumption when heated at 100 °C for 30 minutes is accepted,[23] 400 $\mu$g would be the maximum amount of permanganate used for a 5-ml volume of such a river water. The use of 2 mg of permanganate therefore leaves sufficient reagent to ensure reproducible oxidation of the glycols to their corresponding aldehydes.

An increase in the time of oxidation at 100 °C indicated a decrease in response to each aldehyde precursor. This effect can be attributed to the progressive loss of the aldehydes formed as a consequence of their volatility at elevated temperatures. The change in response to di- and triethylene glycols was small between 3 and 7 minutes' reaction time and hence an optimum time of 5 minutes was chosen for oxidation at 100 °C.

A single addition of sulphuric acid and permanganate gave inconsistent results in preliminary trials. This inconsistency was partly caused by the instability of permanganate

### TABLE II

#### EFFECT OF VARYING CONDITIONS FOR OXIDATION OF GLYCOLS TO ALDEHYDES

| | Optical density ($\times 1000$)* | | | | | |
|---|---|---|---|---|---|---|
| | Monoethylene glycol/mg l$^{-1}$ | | Diethylene glycol/mg l$^{-1}$ | | Triethylene glycol/mg l$^{-1}$ | |
| Variable | 2 | 4 | 2 | 4 | 2 | 4 |
| Amount of permanganate/mg | | | | | | |
| 0·50 | 232 | 472 | 73 | 166 | 102 | 217 |
| 1·00 | 257 | 511 | 96 | 188 | 120 | 233 |
| 1·50 | 254 | 509 | 103 | 202 | 117 | 236 |
| 2·00 | *260* | *520* | *106* | *212* | *118* | *236* |
| Time of oxidation†/ minutes | | | | | | |
| 1 | 303 | 605 | 95 | 184 | 132 | 259 |
| 3 | 280 | 566 | 109 | 218 | 129 | 254 |
| 5 | *260* | *520* | *106* | *212* | *118* | *236* |
| 7 | 248 | 486 | 108 | 214 | 114 | 245 |
| 10 | 219 | 441 | 100 | 204 | 111 | 220 |
| Sulphuric acid concentration‡/N | | | | | | |
| 2 | 298 | 596 | 110 | 220 | 124 | 260 |
| 3 | 271 | 528 | 102 | 206 | 120 | 242 |
| 4 | *260* | *520* | *106* | *212* | *118* | *236* |
| 5 | 222 | 466 | 87 | 180 | 100 | 218 |
| 6 | 220 | 458 | 87 | 185 | 104 | 210 |
| Sulphuric acid concentration (effect on MBTH reaction)§/N | | | | | | |
| 4 | 269 | 547 | 105 | 203 | 120 | 237 |
| 6 | 220 | 463 | 94 | 174 | 111 | 216 |
| Volume of 0·07 M arsenite¶/ml | | | | | | |
| 0·50 | 261 | 534 | 107 | 231 | 120 | 247 |
| 0·75 | 263 | 538 | 108 | 215 | 124 | 248 |
| 1·0 | *260* | *520* | *106* | *212* | *118* | *236* |
| 1·25 | 250 | 520 | 106 | 218 | 119 | 239 |
| 1·50 | 261 | 530 | 99 | 203 | 114 | 235 |

\* Values in italic type are averages.
† Oxidation at 100 °C with 2 mg of permanganate.
‡ Volume = 0·5 ml.
§ Volume = 0·5 ml; reaction after oxidation with 0·5 ml of 2 N acid.
¶ Effect of use of various volumes of 0·07 M arsenite to reduce the excess of permanganate.

in an acidic medium, with decomposition to manganese(IV), and sulphuric acid was therefore added before the permanganate.  As the sulphuric acid concentration increased so the response of each aldehyde precursor decreased.  Subsequent variation of this acid concentration in the reaction stage with MBTH reagent, while maintaining the acid concentration at a constant level during oxidation, indicated that acidity is a factor relevant only to the final reaction stages.  The change in response was small for the addition of 0·5 ml of 3 or 4 N sulphuric acid and the latter concentration was selected.

The removal of excess of permanganate with arsenite was possible over a relatively wide range of sodium arsenite concentrations and 1 ml of 0·07 M arsenite was finally used in the procedure.  This feature was singular, and the need for careful control of reagent additions, and, in particular, the time allowed for oxidation with permanganate must be emphasised in view of previous comments.

Different batches of MBTH reagent showed no significant difference in response to each aldehyde precursor, and similarly no difference in sensitivity was apparent when reagents were prepared or dilutions made with demineralised or distilled water.  Ageing of reagent solutions (over a period of 7 days) produced no change in response but the reagent blank increased with the age of the reagent; for freshly prepared reagent solutions, the reagent blank should have an optical density not exceeding 0·080.  Calibration graphs of optical density against concentration of glycol were linear for the range 0 to 6 mg l$^{-1}$ for each glycol, enabling measurements to be made in the range 0 to 5 mg l$^{-1}$.  Typical optical density responses for each glycol, measured by the procedure described, were: monoethylene glycol, 0·130; diethylene glycol, 0·053; and triethylene glycol, 0·059 per mg l$^{-1}$.

PRODUCTS OF OXIDATION—

The molar extinctions of the final chromogenic solutions, obtained by the procedure outlined, were 8060, 5620 and 8850 for mono-, di- and triethylene glycols, respectively.  From results obtained for formaldehyde,[18] a molar extinction of $1 \times 10^5$ would be expected if complete cleavage of monoethylene glycol to formaldehyde had occurred.  Normal oxidation of the glycols would be expected to yield dialdehydes, e.g., glyoxal (biformyl) would be the expected product from monoethylene glycol.  Other possible products would be glyoxylic acid (oxoacetic acid), glycollic aldehyde (hydroxyacetaldehyde) and glycollic acid (hydroxy-acetic acid) or oxalic acid; for the latter two, no aldehyde response would be obtained.  A molar extinction of 28 000 has been recorded for glyoxal.[16]  We have found that the reaction of MBTH reagent with glyoxal solutions, prepared from both the polymerised monohydrate and from a 40 per cent. m/m solution, gave a response, at concentrations of less than 1 mg l$^{-1}$, corresponding to that obtained by this procedure for monoethylene glycol.  Reaction of MBTH with glyoxylic acid monohydrate, however, gave a molar extinction of 59 000, while glycollic aldehyde gave a molar extinction of 36 500.  It is emphasised that organic reactions that involve oxidation will invariably give several reaction products, but this evidence suggests that the dialdehyde is the major product of oxidation from mono-ethylene glycol for the conditions defined in this method.  It is not certain whether both aldehyde groups react with 2 mol of MBTH initially to give a diazine.  A maximum response is, however, obtained with 1 ml of 1·5 per cent. reagent (Table I), representing a very large excess of reagent, whereas previous applications of MBTH to a wide range of aldehydes and aldehyde precursors have involved the use of 1 ml of 0·8 per cent. reagent[19,21]; it is not clear whether the latter was the over-all optimum concentration.

The molar extinction obtained for diethylene glycol is lower than that obtained for the other two glycols.  This effect could arise because of the formation of a monoazine with MBTH reagent, or the presence, after permanganate oxidation, of one aldehyde group only.  The relatively slow increase to a maximum response with increasing amounts of permanganate (Table II) suggests that labile oxidation of one hydroxyl group via the aldehyde to a carboxylic acid group is unlikely.  Experimental evidence is inadequate to decide which of the pair of alternatives is the cause of this low molar extinction; it is sufficient that a constant level of response is attainable.

SURFACE-WATER SAMPLE BLANKS—

Typical water sample blanks, expressed in terms of optical densities, including and excluding the permanganate oxidation stage, are illustrated in Table III.  The good agreement

obtained indicates the general absence of a natural level of aldehydes, which would be further oxidised by permanganate, and equally, natural levels of aldehyde precursors (*i.e.*, hydroxyl compounds containing primary alcoholic groups). Exceptions to this agreement were noted for domestic effluents. While this agreement holds within our experience, there is a possibility that a surface water could prove anomalous because of polluting species. In such an instance, a sample of the surface water immediately before contamination would be required in order to ascertain the surface-water sample blank with permanganate oxidation. During the monitoring, over a period of 6 months, of river G with a high constant water flow, this sample blank was consistent (Table III), but for other river systems subject to large fluctuations in river flow it might vary with the extent of floodwater, *e.g.*, rivers C and D (Table III). In our experience, volatile alcohols associated with de-icing glycols are seldom encountered in airfield run-offs at water temperatures of greater than 0 °C, because of their volatility, and would not be expected in receiving waters.

TABLE III

COMPARISON OF WATER SAMPLE BLANK VALUES WITH AND WITHOUT THE
PERMANGANATE OXIDATION STAGE

| | Optical density ($\times 1000$) | |
| --- | --- | --- |
| Sample | With oxidation | Without oxidation |
| Spring water    ..    ..    ..    .. | 0 | 0 |
| Well water    ..    ..    ..    .. | 21 | 29 |
| Treated swimming-pool water ..    .. | 35 | 37 |
| River water: A ..    ..    ..    .. | 7 | 0 |
| B   ..    ..    ..    .. | 35 | 29 |
| C, October, 1972 ..    .. | 32 | 37 |
| C, November, 1972    .. | 75 | 65 |
| D, April, 1972    ..    .. | 40 | 45 |
| D, October, 1972 ..    .. | 112 | 104 |
| Estuary water E ..    ..    ..    .. | 77 | 69 |
| River water: F ..    ..    ..    .. | 107 | 105 |
| G, June, 1972    ..    .. | 90 | 80 |
| G, August, 1972 ..    .. | 84 | 89 |
| G, November, 1972    .. | 96 | — |
| G, December, 1972    .. | 95 | — |
| Airfield ditch H ..    ..    ..    .. | 40 | 34 |
| Airfield ditch J ..    ..    ..    .. | 140 | 132 |
| Domestic sewage effluent    ..    .. | 127 | 76 |
| Partially treated domestic sewage    .. | 107 | 55 |

A SENSITIVE VARIATION FOR DETERMINATION OF MONOETHYLENE GLYCOL
INVOLVING CLEAVAGE TO FORMALDEHYDE

Cleavage with periodic acid of vicinal diols followed by reaction with chromotropic acid of the aldehydes formed,[13] and similarly, cleavage of molecules containing the 2-aminoethanol and ethylenediamine fragments to aldehydes and subsequent measurement with MBTH reagent, have been described.[20] A similar oxidation of monoethylene glycol would yield formaldehyde, which would give a coloured cation with a high molar extinction with MBTH reagent. Calculation from values of optical densities obtained previously for formaldehyde[18] indicates that an optical density of 0·660 per mg l⁻¹, measured at 630 nm in 1-cm cells, would be expected from the splitting of monoethylene glycol and subsequent measurement in a final 25-ml volume of solution. In practice, water sample blank values with and without periodate oxidative cleavage disagreed (Table IV). This disagreement could be explained by the extreme sensitivity of the reaction conditions to polyhydroxyaldehyde precursors, involving both primary and secondary alcoholic groups, which cleaved to form formaldehyde or aldehydes of low relative molecular mass of similar sensitivity. This effect must be compared with the absence of aldehyde precursors for acidified permanganate as the oxidation medium, when only primary alcoholic groups are involved. Allowing for this inherent weakness, which implies that monitoring a surface water would require sample blank values before contamination, the extreme sensitivity of this variation commends it for attention.

A brief discussion and an outlined procedure is therefore included below for information.

TABLE IV

WATER SAMPLE BLANK VALUES WITH AND WITHOUT PERIODATE OXIDATION

| River water | | | | Optical density ($\times 1000$) | | | |
|---|---|---|---|---|---|---|---|
| | | | | C | D | F | G |
| With periodate.. | .. | .. | | 19 | 153 | 254 | 96 |
| Without periodate | .. | .. | | 0 | 35 | 40 | 27 |

DISCUSSION—

The results for different oxidative reagent conditions are shown in Table V. Complete reaction at room temperature was uncertain and results indicated that no disadvantage accrued from heating, consistent response being achieved for 1 to 8 minutes' reaction time at 100 °C; a time of 2 minutes was chosen for this reaction stage. There were no restrictions on oxidation for initial volumes of 0 to 11 ml of solution, thus enabling the monoethylene glycol content of 10-ml samples of surface waters to be determined. Variation of the sulphuric acid concentration for the MBTH reagent stages showed that as acidity increased, sensitivity decreased. It was found that the acid used, 1 ml of 2 N sulphuric acid, was not essential for the initial oxidative cleavage, but it was convenient to use acidified periodate as it increased the solubility of potassium periodate in a cold aqueous solution and reduced the number of solutions that had to be added.

Variation in the MBTH reagent concentration indicated that reaction with 1 ml of 2 per cent. MBTH reagent at 100 °C was required; constant response was obtainable for a reaction time of 4 to 8 minutes and 6 minutes was accepted as the optimum time. The response to the addition of the 2 per cent. iron(III) chloride - 3 per cent. sulphamic acid reagent was a maximum for the addition of 1·0 to 1·25 ml of reagent, stable readings being obtained 15 to 20 minutes after addition. For smaller volumes of the composite reagent, a lower response was obtained, while for larger volumes, fading of the final blue cationic

TABLE V

EFFECT OF VARYING REAGENT CONDITIONS FOR PERIODATE OXIDATION OF
MONOETHYLENE GLYCOL

| | | Optical density ($\times 1000$) | | |
|---|---|---|---|---|
| Monoethylene glycol concentration/mg $l^{-1}$ .. | | 0·2 | 0·6 | 1·0 |
| Reaction conditions with 1 ml of 0·04 M periodate | | | | |
| Temperature | Time/minutes | | | |
| Ambient | 5 | 90 | 276 | 500 |
| | 10 | 114 | 334 | 564 |
| | 15 | 133 | 387 | 669 |
| | 20 | 141 | 386 | 661 |
| | 30 | 133 | 385 | 662 |
| 100 °C | 1 | 144 | 414 | 669 |
| | 2 | 140 | 412 | 667 |
| | 4 | 144 | 417 | 674 |
| | 8 | 139 | 417 | 675 |
| Sulphuric acid concentration*/N | | | | |
| 2 | | 129 | 404 | 674 |
| 3 | | 134 | 394 | 672 |
| 4 | | 123 | 370 | 615 |
| MBTH concentration,† per cent. | Time/minutes | | | |
| 0·5 | 4 | 41 | 160 | 280 |
| 1·0 | 4 | 89 | 307 | 485 |
| 2·0 | 2 | 111 | 311 | 541 |
| 2·0 | 4 | 139 | 404 | 671 |
| 2·0 | 8 | 140 | 414 | 671 |

* Volume of acid = 1 ml.
† Volume of reagent = 1 ml.

chromogen occurred within this time span. A volume of 1 ml of composite reagent was therefore selected and the final colour was read 20 minutes after its addition.

By using the procedure described, a molar extinction of $1.05 \times 10^5$ is obtained for mono-ethylene glycol, which compares favourably with the value of $7.3 \times 10^4$ obtained previously in a general method for polyhydroxyaldehyde precursors.[21]

REAGENTS—

These should be of analytical-reagent grade when available; solutions can be prepared with de-ionised water.

*Sulphuric acid,* 2 N.

*Potassium periodate solution,* 0·04 M—Dissolve 0·92 g of potassium periodate in 100 ml of 2 N sulphuric acid.

*Sodium arsenite solution,* 1 M—Dissolve 13 g of the reagent in 100 ml of water.

*3-Methylbenzothiazol-2-one hydrazone hydrochloride (MBTH), 2 per cent.* m/V *solution*—Dissolve 2 g of the reagent in 100 ml of water.

*Iron(III) chloride - sulphamic acid solution*—Dissolve 2 g of iron(III) chloride hexa-hydrate and 3 g of sulphamic acid in water and dilute the mixture to 100 ml.

*Standard monoethylene glycol solution*—Mix 10 g of the glycol with 1 litre of water to give a solution containing 10 000 mg $l^{-1}$. Immediately prior to use, dilute 50 ml of this standard solution to 1 litre to give a solution containing 500 mg $l^{-1}$; 2 ml of this solution diluted to 500 ml gives a working solution of 2 mg $l^{-1}$.

PROCEDURE—

Measure 10 ml of settled sample (or sample after filtration through pre-washed cotton-wool), of a suitable aliquot diluted to 10 ml, into a 25-ml calibrated flask, and also prepare a reagent blank with 10 ml of de-ionised water. Add 1 ml of periodate reagent to each flask, mix the contents well and immerse in a boiling water bath for 2 minutes. Withdraw the flasks from the water-bath and remove excess of periodate by reaction with 1 ml of sodium arsenite, then add 1 ml of MBTH reagent solution and immerse them in the water-bath for a further 6 minutes. Remove and cool to room temperature, add by pipette 1 ml of iron(III) chloride - sulphamic acid reagent to each flask and dilute the contents to 25 ml. Stand for 20 minutes and read the optical density at 630 nm in a clean 1-cm cell with water in the reference cell. The net optical density for the sample is obtained by subtracting the reagent blank.

To compensate for interference from the natural levels of oxidisable material in surface waters, a sample blank value before contamination must be obtained. The optical density due to monoethylene glycol, obtained by subtracting this natural blank value from the net sample optical density, can be expressed as concentration of glycol by reference to a cali-bration graph prepared by diluting 0, 1, 2, 3, 4 and 5 ml of standard solution containing 2 mg $l^{-1}$ of glycol to 10 ml with de-ionised water in 25-ml calibrated flasks. A graph of the optical densities, less reagent blank, plotted against the concentration of glycol is linear for the range 0 to 1 mg $l^{-1}$, with an optical density of 0·680 per mg $l^{-1}$ for monoethylene glycol.

### RESULTS OF RECOVERY EXPERIMENTS FOR MONO-, DI- AND TRIETHYLENE GLYCOLS

Results of recovery experiments, involving the concentration of surface-water samples, on a water-bath, suggest that attempts to improve sensitivity by this means cannot be recom-mended. While satisfactory recovery and precision were obtained for uncontaminated water samples, river-water samples gave excessive recovery and low accuracy. This result could be explained by possible biodegradation, in particular of monoethylene glycol, and in part by the inconsistencies of the water sample blanks on concentration. Sample blanks measured after concentration and permanganate oxidation increased according to the degree of concentration. Similar blanks obtained without oxidation were disproportionate to the extent of concen-tration; this disparity could be due to aerial oxidation of the organic constituents to aldehydes during the evaporation.

The results of recovery experiments for each glycol in the range 1 to 5 mg $l^{-1}$, with use of the acidified permanganate procedure, are shown in Table VI. For each glycol, 5 ml of each of 20 and 100 mg $l^{-1}$ solutions were diluted to 100 ml with each of the filtered waters indicated. Allowance was made, when necessary, for the water sample blank to account for dilution of water sample with standard. The freshly collected river waters were of varying

TABLE VI
RECOVERY OF GLYCOLS FROM FORTIFIED SAMPLES

| | Permanganate value/ mg l$^{-1}$ | Monoethylene glycol/mg l$^{-1}$ | | Diethylene glycol/mg l$^{-1}$ | | Triethylene glycol/mg l$^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | | 1 | 5 | 1 | 5 | 1 | 5 |
| Spring water.. .. | 0·4 | 0·90 | 5·05 | 1·05 | 5·10 | 1·11 | 4·94 |
| Well water .. .. | 1·8 | 1·03 | 5·23 | 0·85 | 4·63 | 1·00 | 4·89 |
| Public water supply | 1·4 | 0·94 | 4·89 | 1·17 | 4·63 | 1·05 | 5·02 |
| River water: A .. | 2·0 | 1·02 | 5·19 | 1·07 | 5·20 | 1·03 | 4·84 |
| C .. | 7·0 | 0·84 | 4·87 | 1·09 | 4·78 | 0·98 | 5·32 |
| D .. | 2·6 | 0·98 | 5·11 | 0·91 | 4·97 | 1·11 | 4·80 |
| G .. | 6·0 | 1·19 | 5·08 | 0·92 | 4·70 | 1·00 | 5·20 |
| G .. | 6·0 | 0·97 | 5·04 | 1·06 | 5·34 | 1·02 | 5·18 |

composition from different topographical origins. Rivers A and C originated as upland streams, while rivers D and G were slow-flowing rivers passing through agricultural land into which sewage effluents discharged. The average recovery was 100·5 per cent. with an over-all coefficient of variation of 7 per cent. At the 1 mg l$^{-1}$ level this value was 9 per cent., while at the 5 mg l$^{-1}$ level it was 4 per cent. Based on these coefficients of variation and those of reagent and sample blanks, a limit of sensitivity of the order of 0·5 mg l$^{-1}$ was indicated. Recoveries from estuary water E, with dissolved solids at a concentration of 15 300 mg l$^{-1}$, were low and are not included; this low recovery suggests that the method would be invalid for saline waters.

The method has been used successfully in these laboratories to monitor the biodegradation of low levels of glycols in river waters.

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# A Spectrophotometric Method for the Micro-determination of Piperonyl Butoxide in the Presence of Pyrethrins

By H. M. BHAVNAGARY AND S. M. AHMED

(*Central Food Technological Research Institute, Mysore-2A, India*)

A rapid spectrophotometric method has been developed for the micro-determination of piperonyl butoxide in the presence of a wide range of pyrethrins. A solution of the mixture of piperonyl butoxide and pyrethrins in a low-boiling fraction of light petroleum is evaporated on a water-bath until only a small portion of the solvent remains. The final traces of solvent are removed at 50 °C and, to the residue containing piperonyl butoxide and pyrethrins, 18 per cent. $m/V$ nitric acid is added in order to convert the piperonyl butoxide content into a soluble yellow-coloured compound. The quantitative colour reaction has a maximum absorption at 370 nm, and the method is applicable to the residues in the range 4 to 40 $\mu$g ml$^{-1}$ of piperonyl butoxide. The method is also applicable to the residues of formulated products extracted from grains and paper coatings.

PIPERONYL butoxide, 5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole,[1] has been widely used in combination with pyrethrins as synergist in various formulations for the protection of grains and foodstuffs, in the control of household insect pests, in food processing plants and warehouses, and for fish preservation. The use of piperonyl butoxide has increased in recent years not only because of its synergism[2] with pyrethrins but also because of the low toxicity of such combinations to warm-blooded animals.[3] A survey of the literature revealed that the gas-chromatographic methods[4,5] for the quantitative determination of piperonyl butoxide involved preliminary clean-up procedures. Jones, Ackermann and Webster[6] have reported a colorimetric method for the determination of piperonyl butoxide alone in which they heated it in purified kerosene with a solution of tannic acid in a mixture of phosphoric and glacial acetic acids in order to produce a quantitative blue colour reaction. This method could not be used in the presence of pyrethrins owing to interference by the latter. In an attempt to avoid this interference, they saponified the pyrethrins content of the mixtures before colour development, but this treatment reduced the colour value. They then suggested that piperonyl butoxide could be separated from pyrethrins by using the partition-chromatographic method. The interference of pyrethrins in the above method was also observed by Williams, Dale and Sweeney,[7] who considered that it was caused by the red colour produced in the reaction that occurred between the phosphoric acid and pyrethrins. Further, the method of Jones *et al.* was used by other workers[8,9] only after they had isolated the piperonyl butoxide by means of a column-chromatographic separation in order to prevent interference by the pyrethrins. This procedure again involved the use of additional steps, thus making the method more time consuming and increasing the error. A need was therefore felt to develop a simple spectrophotometric method for the determination of piperonyl butoxide in the presence of pyrethrins.

A quantitative colour reaction of nitric acid with piperonyl butoxide has accordingly been developed that forms the basis of a method for its determination. The method is highly sensitive and the coloured compound formed obeyed Beer's law for a suitable range of micro-amounts of piperonyl butoxide down to a minimum concentration of 4 $\mu$g ml$^{-1}$. The method is free from interference by pyrethrins.

## METHOD

APPARATUS—

*Spectrophotometer*—Beckman DU model, with a 10-mm cell.

*Colorimeter*—Bausch and Lomb Spectronic 20, with 12 × 100-mm tubes. For convenience, measurements were made at 375 nm rather than at the wavelength of maximum absorption (370 nm).

*Test-tubes*—Ground-glass, stoppered test-tubes, with the dimensions 18 × 150 mm, were used for the colour reaction.

REAGENTS—

*Piperonyl butoxide*—Technical material with a minimum content of 80 per cent. of piperonyl butoxide.

*Nitric acid reagent*—Add 250 ml of analytical-reagent grade nitric acid (sp. gr. 1·42) to sufficient glass-distilled water to give 1 litre of solution.

*Pyrethrum extract*—A standardised commercial extract containing 20 per cent. *m/m* of pyrethrins.

*Light petroleum*—Extra pure, with boiling range 40 to 60 °C, and free from aromatic compounds, as supplied by Reechem (RC, India).

PREPARATION OF STANDARD SOLUTION—

For the stock solution, dissolve 0·25 g of technical piperonyl butoxide in light petroleum and make the solution up to 250 ml in a standard flask. With a pipette, transfer 10 ml of the solution into a 100-ml standard flask and make the volume up to the mark with light petroleum, thus giving a solution containing 100 $\mu$g ml$^{-1}$ of piperonyl butoxide.

PROCEDURE—

*Preparation of calibration graph*—Transfer 0, 0·2, 0·4, 0·6, 0·8, 1·0, 1·2, 1·4, 1·6, 1·8 and 2·0 ml of the standard solution into glass-stoppered test-tubes and heat them on a water-bath until the residue obtained is solvent free. Add exactly 5 ml of the nitric acid reagent by means of a pipette and shake the mixture for 1 minute at a temperature not higher than 35 °C. Then measure the absorbance of the solution against a blank solution in the 12 × 100-mm tubes of the Bausch and Lomb colorimeter at 375 nm. Prepare a calibration graph from the results obtained.

*Preparation of test samples*—Dissolve an accurately weighed amount of the samples containing pyrethrins and piperonyl butoxide in various proportions, such as 1 to 3, 1 to 5, 1 to 10 and 1 to 15, in light petroleum so as to give solutions that have piperonyl butoxide contents in the range 5 to 50 $\mu$g ml$^{-1}$. Also, dissolve in the same solvent known amounts, within the above range, of piperonyl butoxide alone.

COLORIMETRIC DETERMINATION—

Transfer an aliquot of 5 ml of the test samples into 18 × 150-mm glass-stoppered test-tubes. Evaporate the solution on a water-bath until a small portion of the solvent remains, then continue to evaporate it in a water-bath adjusted to 50 °C until all of the solvent has been removed. To the residue add exactly 5 ml of the colour-forming reagent, stopper the test-tube and shake it well for 1 minute. Treat 5 ml each of the known sample and reagent blank in a similar manner. Then transfer the coloured solutions to the Bausch and Lomb colorimeter tubes and read the absorbance at 375 nm. Make all determinations in duplicate.

CALCULATION OF RESULTS—

The piperonyl butoxide content of the sample is calculated as follows.

$$\text{Piperonyl butoxide content} = \frac{W(c-a)}{(b-a)} \, \mu\text{g}$$

where $W$ $\mu$g is the amount of piperonyl butoxide in the known sample, $a$ the absorbance of the blank, $b$ the absorbance of the known sample and $c$ the absorbance of the test solution.

DISCUSSION

COLOUR REAGENT—

Under normal conditions, phenolic ethers undergo sulphonation, nitration and chlorination[10] with electrophilic reagents by substitution in the ring. These reactions were carried

out on piperonyl butoxide by using the appropriate concentrated acids. It was observed that the reaction with nitric acid was quantitative and gave a yellow-coloured nitro compound that was soluble in the excess of nitric acid. Further investigations with dilute nitric acid revealed that the reaction was complete when nitric acid in the concentration range 10 to 18 per cent. $m/V$ was used for the nitration in aqueous medium.

CHARACTERISTICS OF THE COLOUR REACTION—

The absorption spectrum of the yellow-coloured nitrated piperonyl butoxide was determined on the Beckman DU spectrophotometer in the wavelength range from 340 to 440 nm. Fig. 1 shows the spectra obtained with 30 and 60 $\mu$g of piperonyl butoxide present in 1 ml of solution, the maximum absorption occurring at wavelength 370 nm. The relationship between the concentration and the colour intensity obeyed Beer's law in the range 4 to 40 $\mu$g ml$^{-1}$, the graph obtained being a straight line that passed through the origin. For the preparation of the standard graph, both technical and purified grades of piperonyl butoxide can be used as there was no quantitative difference between the colour reactions. The samples of piperonyl butoxide used should be free from organic solvents such as light petroleum, trichloroethylene and benzene, as the nitro compound partitions between these solvents and nitric acid.
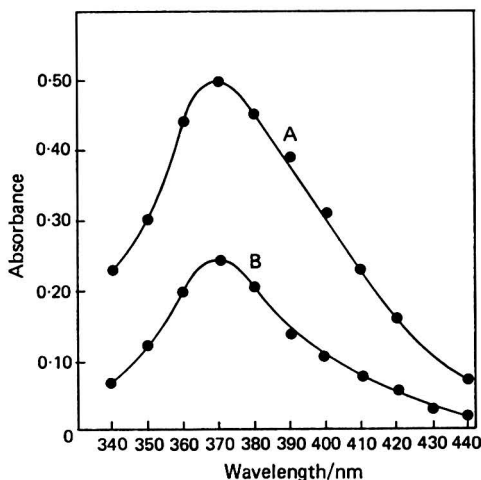


Fig. 1. Absorption spectra of piperonyl butoxide - nitric acid compound with reagent blank containing 14 per cent. of nitric acid: A, 60 $\mu$g ml$^{-1}$ of piperonyl butoxide; and B, 30 $\mu$g ml$^{-1}$ of piperonyl butoxide

STABILITY OF THE COLOURED COMPOUND IN THE TEMPERATURE RANGE 25 TO 30 °C—

The stability of the coloured product developed with 20 and 37 $\mu$g of piperonyl butoxide per millilitre of nitric acid solution was studied by reading the absorbances at 375 nm at intervals of 1 minute for a period of 10 minutes and then at intervals of half an hour for a period of 10 hours. It was found to be stable for a period of up to 10 hours, beyond which time there was a gradual deterioration, as shown in Table I.

EFFECT OF HEAT ON THE COLOUR REACTION—

The absorbances of the colour developed at various temperatures between 20 and 100 °C with 165 $\mu$g of piperonyl butoxide, by adding 5 ml of the reagent, were studied. The relationship between the temperature of reaction and the absorbance at 375 nm is shown in Fig. 2. The coloured compound was stable up to a temperature of 35 °C, above which it decomposed steadily with increase in temperature. It is therefore necessary to maintain the temperature of the reaction mixture below 35 °C for satisfactory colour development.

TABLE I

STABILITY OF THE COLOURED COMPOUND IN THE TEMPERATURE RANGE 25 TO 30 °C

| Time after development | Absorbance* | | Time after development | Absorbance* | |
|---|---|---|---|---|---|
| | 20 $\mu g$ ml$^{-1}$ of piperonyl butoxide | 37 $\mu g$ ml$^{-1}$ of piperonyl butoxide | | 20 $\mu g$ ml$^{-1}$ of piperonyl butoxide | 37 $\mu g$ ml$^{-1}$ of piperonyl butoxide |
| 1 minute | 0·12 | 0·22 | 10 hours | 0·12 | 0·22 |
| 5 minutes | 0·12 | 0·22 | 12 hours | 0·11 | 0·21 |
| 10 minutes | 0·12 | 0·22 | 15 hours | 0·10 | 0·19 |
| 30 minutes | 0·12 | 0·22 | 18 hours | 0·09 | 0·17 |
| 1 hour | 0·12 | 0·22 | 21 hours | 0·08 | 0·15 |
| 5 hours | 0·12 | 0·22 | 24 hours | 0·07 | 0·14 |

* Measured at 375 nm in 12-mm tubes against water as reference.

EFFECT OF THE NITRIC ACID REAGENT CONCENTRATION—

To test-tubes each containing 150 $\mu g$ of piperonyl butoxide, 5 ml of nitric acid at different concentrations were added and the colour was developed by shaking the mixture for 1 minute. In Fig. 3, the relationship between the concentration of nitric acid and the absorbance is shown. With dilute acids the colour reaction with piperonyl butoxide was incomplete. At an acid concentration of 10 per cent. $m/V$ the absorbance reached a maximum and thereafter remained constant in the range 10 to 18 per cent. $m/V$, indicating that the colour reaction was complete. The concentration of the nitric acid used as the colour-forming reagent should therefore be above 10 per cent. $m/V$.
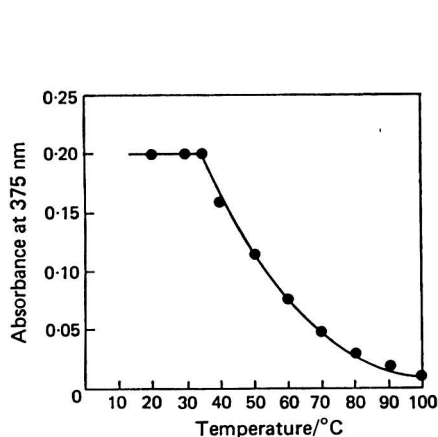


Fig. 2.  Effect of temperature on the absorbance of the piperonyl butoxide - nitric acid coloured compound
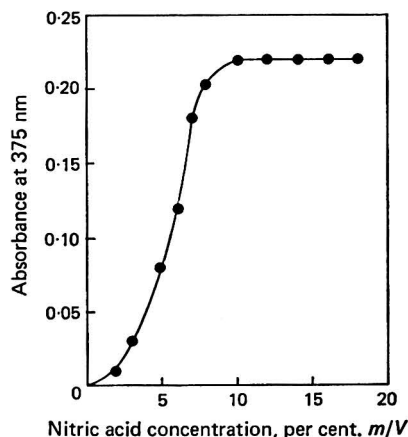
Fig. 3.  Effect of concentration of nitric acid on the absorbance of the coloured compound containing 30 $\mu g$ ml$^{-1}$ of piperonyl butoxide

APPLICATION OF THE METHOD—

The rapid spectrophotometric procedure developed has been applied satisfactorily to formulations containing pyrethrins and piperonyl butoxide in various proportions. It can also be used for the determination after extracting the combinations of pyrethrins and piperonyl butoxide from both sorptive and non-sorptive surfaces, such as glass, grains, paper coatings, etc. Either light petroleum or hexane can be used for the extraction and the solvent-free residues taken for the determination by the procedure described.

CONCLUSIONS

A sensitive and precise method for the determination of piperonyl butoxide in the presence of pyrethrins by a spectrophotometric procedure is presented. It is based on the

measurement of the yellow colour produced when the samples containing piperonyl butoxide are treated with 10 to 18 per cent. $m/V$ nitric acid at a temperature not higher than 35 °C.

The colour is stable and appears to be fairly specific for piperonyl butoxide. There is no interference at any concentration level from pyrethrins, with which it is commonly used in formulations.

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# The Determination of Chlorhydroxyquinoline in Medicated Pig Feeds

By J. E. FAIRBROTHER and W. F. HEYES

*(Squibb International Development Laboratory, Moreton, Wirral, Cheshire)*

A method has been developed for the determination of chlorhydroxy-quinoline (Halquinol) in medicated pig feeds. Because of the interference by feed constituents in simple spectrophotometric and polarographic assay procedures, a spectrofluorimetric procedure is recommended. Spectrofluori-metric measurements are taken in a methanolic solvent containing 5 per cent. of chloroform, and the fluorescence of chlorhydroxyquinoline, as its magnesium chelate, is measured at 500 nm, with an excitation wavelength of 402 nm. Cyanide is used to suppress interference from copper and zinc salts that are commonly added to these feeds. The procedure is not affected by the presence of other feed additives, such as dimetridazole and arsanilic acid.

CHLORHYDROXYQUINOLINE, **I**, is a mixture of three isomeric chloro-8-hydroxyquinolines that is claimed[1] to possess antibacterial and antifungal activities greater than those of its individual components:
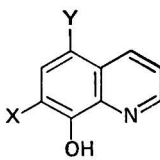


(Halquinol)

where $X = Cl$, $Y = Cl$ (57 to 74 per cent. *m/m*); $X = H$, $Y = Cl$ (23 to 40 per cent. *m/m*); and $X = Cl$, $Y = H$ (not more than 3 per cent. *m/m*).

One of the veterinary uses of chlorhydroxyquinoline is in the treatment of bacterial diarrhoea in weanling pigs. The drug is marketed for this purpose as a pre-mix ("Quixalud," E. R. Squibb & Sons Ltd.) that is diluted with pig feed.

Chlorhydroxyquinoline is readily soluble in chloroform and shows an absorption maximum at 335 nm ($E_{1cm}^{1\%}$ = about 150). It forms chelates with many cations, in a number of instances (iron, copper, vanadium, titanium and molybdenum) giving coloured products suitable for spectrophotometric determination. The green chelate formed with iron(III) (with an absorption maximum at 685 nm) is soluble in a mixture of chloroform and acetone and can be used (J. E. Fairbrother and W. F. Heyes, unpublished work) for the spectrophotometric determination of chlorhydroxyquinoline in pre-mixes that contain inorganic diluents.

The determination of chlorhydroxyquinoline in medicated pig feeds, however, is difficult for two reasons. Firstly, simple solvent extraction in order to remove the active substance from the feed also removes feed constituents that produce a high level of background absorption in both the ultraviolet and visible-light regions of the spectrum. Secondly, in polarographic procedures (J. E. Fairbrother and W. F. Heyes, unpublished work), constituents of medicated feeds either interfere in the assay or prevent the quantitative extraction of chlorhydroxyquinoline. We therefore decided to examine a spectrofluorimetric procedure for the determination of chlorhydroxyquinoline in a medicated pig feed that contained 600 g ton$^{-1}$ of chlorhydroxyquinoline (about 590 p.p.m.). The procedure had to be capable of determining the active substance in the presence of other common feed additives, such as copper and zinc salts, dimetridazole and arsanilic acid.

## EXPERIMENTAL

The chlorhydroxyquinoline is extracted from the sample of medicated feed by being shaken with chloroform. After the chloroform extract has been filtered an aliquot is treated with a solution of potassium cyanide in methanol, in order to complex any co-extracted copper and zinc.

A methanolic magnesium acetate reagent solution is added to the treated aliquot to convert the chlorhydroxyquinoline into its fluorescent chelate with magnesium and the solution is diluted with neutralised methanol containing phenolphthalein indicator. The alkalinity of this solution is adjusted to a defined point by the dropwise addition of methanolic potassium hydroxide solution, then the fluorescence-emission intensity of the solution is measured and compared quantitatively with that of a similarly treated chlorhydroxyquinoline standard.

## METHOD

### APPARATUS—

A Baird Atomic SF1 spectrofluorimeter was used, with exit and entrance slits set at 8 $\mu$m. The scale used (coarse gain, 1000 at 1 s; fine gain, 10; photomultiplier setting, 3 or 4) was adjusted to give a meter reading of 60 for the chlorhydroxyquinoline standard solution. The spectrofluorimeter was coupled with an Advance Electronics $XY$ pen recorder, Model HR 100, and recorded spectra rather than direct meter readings were used.

### REAGENTS—

All reagents were of analytical-reagent grade, unless otherwise stated.

*Chlorhydroxyquinoline*—A sample of the batch used to medicate the feed, or a suitable reference standard.

*Chloroform.*

*Magnesium acetate solution*—A 0·1 per cent. $m/V$ solution in methanol.

*Methanol, neutralised*—With 0·1 M methanolic potassium hydroxide solution, neutralise 500 ml of methanol containing 0·75 ml of 1·0 per cent. $m/V$ phenolphthalein solution to a faint pink colour.

*Phenolphthalein solution*—A 1·0 per cent. $m/V$ solution in ethanol.

*Potassium cyanide solution*—A 0·5 per cent. $m/V$ solution in methanol.

*Potassium hydroxide solution, 0·1 M*—Weigh 0·561 g of potassium hydroxide into a 100-ml calibrated flask. Dissolve it in, and dilute to volume with, methanol.

### PREPARATION OF STANDARD—

Weigh accurately 120 mg of chlorhydroxyquinoline into a 100-ml calibrated flask. Dissolve it in, and dilute to volume with, chloroform. Mix the solution thoroughly and transfer 10 ml by pipette into another 100-ml calibrated flask, dilute to volume with chloroform, and again mix thoroughly.

### PREPARATION OF SAMPLE—

Weigh accurately 20 g of medicated feed (or an amount containing approximately 12 mg of chlorhydroxyquinoline) into a 250-ml conical flask. Add, by use of a pipette, 100 ml of chloroform, then stopper the flask and shake it thoroughly for 5 minutes. Immediately filter about 20 ml of this solution through a fluted Whatman No. 4 filter-paper, rejecting the first 10 ml of filtrate.

### REACTION PROCEDURE—

Transfer, with a pipette, 5 ml of each of the standard and sample solutions into separate 100-ml calibrated flasks and treat each in the following manner. With care add, by use of an automatic pipette, 5 ml of potassium cyanide solution followed by 10 ml of magnesium acetate solution. Mix the solutions and add about 60 ml of neutralised methanol containing phenolphthalein indicator. Then, by using a Pasteur pipette, add 0·1 M methanolic potassium hydroxide solution dropwise until the solution just turns pink. Add a further 1·5 ml of 0·1 M methanolic potassium hydroxide solution from a pipette and dilute each solution to volume with more neutralised methanol. Mix them thoroughly, then filter, if necessary, through a Whatman No. 1 filter-paper, rejecting the first 10 ml of filtrate.

SPECTROFLUORIMETRIC MEASUREMENT—

Scan the fluorescence-emission spectrum of a 1-cm layer of both the sample and standard solutions between 485 and 570 nm, with an excitation wavelength of 402 nm. (The emission peak should occur at approximately 500 nm.) Record the peak heights given by the sample and standard solutions, and calculate the chlorhydroxyquinoline content of the sample. If necessary, a sample of unmedicated feed should be put through the procedure as described for the sample, and a suitable blank correction should be made to the reading for the sample of feed.

## RESULTS AND DISCUSSION

REACTION CONDITIONS—

Inadequate recoveries of chlorhydroxyquinoline obtained in initial experiments were found to have resulted from a reduction in fluorescence yield, rather than from inefficient extraction from the feed. This result was found to correlate with the co-extraction of acidic components from the feed, and the problem was overcome by controlling the alkalinity of the solution (see Fig. 1). The change in fluorescence yield brought about by the addition of alkali was first measured by use of external acid - base indicators. It was then found that the inclusion of the indicator in the reaction mixture did not significantly affect the results, and in view of the necessity to retain methanol as the main solvent, the use of the internal indicator was adopted.
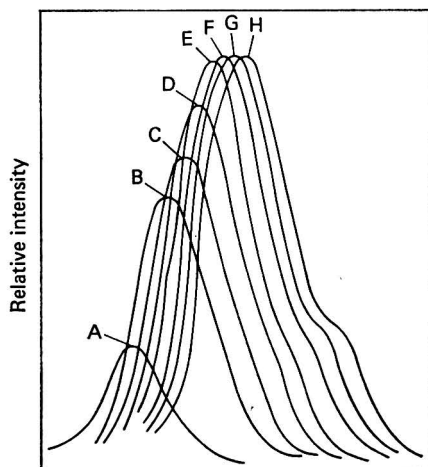


Fig. 1. Fluorescence (emission) spectrum of a methanolic solution of chlorhydroxyquinoline - magnesium complex neutralised with potassium hydroxide: A, to bromothymol blue external indicator; B, to phenolphthalein; and C to H, to phenolphthalein with 0·25 (C), 0·5 (D), 0·75 (E), 1·0 (F), 1·5 (G) and 2·0 (H) ml excess of 0·1 M methanolic potassium hydroxide solution per 100 ml of solution. (Subsequent spectra were recorded off-set with respect to the wavelength scale so as to permit comparison)

A study of the extraction procedure showed that recoveries of chlorhydroxyquinoline from medicated feeds were dependent on time, values reducing to about 70 per cent. recovery after 30 minutes' extraction. An examination of the chloroform extracts from medicated feeds by use of atomic-absorption spectroscopy showed that copper and zinc were the main cations co-extracted with the active drug. These ions were found to compete with magnesium

acetate reagent for chelation with the chlorhydroxyquinoline, thereby reducing the fluorescence intensity of the chlorhydroxyquinoline - magnesium chelate solution. A small amount of extracted manganese ion had no significant effect on the fluorescence. When potassium cyanide reagent was added to the chloroform extract the copper and zinc ions complexed with it rather than with chlorhydroxyquinoline, thus overcoming the extraction problem and that of poor recoveries.

FLUORESCENCE PHENOMENA—

The fluorescence spectra of certain metal chelates of 8-hydroxyquinoline and its derivatives have been extensively reported.[2–8] The fluorescence spectrum of chlorhydroxyquinoline is similar to that of 8-hydroxyquinoline,[3,8] but its excitation and emission maxima are shifted slightly towards longer wavelengths.

The possibility that interfering substances are co-extracted from the feed, as well as the limited availability of solvents of suitable purity, limited the choice of solvents to the chloroform - methanol mixture used.

Aluminium, magnesium and lithium were considered as possible chelating cations, and magnesium was selected. Although aluminium was known[5] to give the highest fluorescence yield, it was difficult to select an aluminium salt soluble in a chloroform - methanol mixture and also available in a sufficiently pure form for spectrofluorimetric work. Lithium had a lower fluorescence intensity than magnesium and was therefore not examined further.

Under the conditions selected, chlorhydroxyquinoline showed an excitation maximum at 402 nm and a corresponding emission maximum at 500 nm. The linearity of the relationship between the emission intensity and the concentration of the chlorhydroxyquinoline solution was demonstrated over the concentration range 3·75 to 7·25 $\mu$g ml$^{-1}$, and the fluorescence intensities of the sample and standard solutions remained reproducible for at least 1 hour after their preparation.

It was considered valuable to examine the fluorescence characteristics of the three isomeric components of chlorhydroxyquinoline. The changes in fluorescence characteristics caused by variations in the ratio of the component isomers were also examined (see Table I).

## TABLE I
### FLUORESCENCE CHARACTERISTICS OF CHLORHYDROXYQUINOLINE AND ITS COMPONENT SUBSTANCES

|  |  | Wavelength of excitation maximum/nm | Wavelength of emission maximum/nm | Relative fluorescence intensity |
|---|---|---|---|---|
| 5,7-Dichloro-8-hydroxyquinoline | .. | 402 | 495 | 79·3 |
| 5-Chloro-8-hydroxyquinoline .. | .. | 402 | 510 | 35·7 |
| 7-Chloro-8-hydroxyquinoline .. | .. | 388 | 487 | 60·2 |
| Chlorhydroxyquinoline—Batch A | .. | 402 | 500 | 64·5 |
| B | .. | 402 | 500 | 64·5 |
| C | .. | 400 | 497 | 62·5 |
| D | .. | 402 | 498 | 64·2 |

Although the fluorescence characteristics of the three component isomers are different, the over-all effect of these differences on the fluorescence yield of different batches of chlorhydroxyquinoline is not critical. However, it is recommended that a sample of the same batch of chlorhydroxyquinoline that was used to medicate the feed be used as a standard in the assay procedure.

## RESULTS

Feed blanks did not appear to make any major contribution to the fluorescence intensity, except for those from feeds that contained a high proportion of meat meal. However, because different feed bases were not studied extensively, such interferences cannot be ruled out for all grades of feed.

Recoveries of chlorhydroxyquinoline from different types of feeds medicated in the laboratory are shown in Table II.

TABLE II

RECOVERY OF CHLORHYDROXYQUINOLINE FROM FEEDS MEDICATED IN THE
LABORATORY AT LEVELS BETWEEN 60 AND 140 PER CENT. OF THE
THEORETICAL CONTENT (600 g ton$^{-1}$)

| Feed sample | Halquinol added/ g ton$^{-1}$ | Feed blank, per cent. of contribution of active drug | Recovery of chlorhydroxyquinoline, per cent. (corrected for blank contribution) |
|---|---|---|---|
| 1 | 600 | 3·3 | 97·8, 97·9, 99·0 |
|   | 360 | 5·4 | 106·2, 104·2 |
|   | 840 | 2·4 | 93·0, 93·8 |
| 2 | 600 | 3·3 | 99·2, 96·6, 96·6 |
|   | 360 | 5·4 | 98·8, 98·9 |
|   | 840 | 2·4 | 97·9, 96·6 |
| 3 | 600 | 3·3 | 100·2, 102·1, 97·1 |
|   | 360 | 5·3 | 102·5, 105·5 |
|   | 840 | 2·4 | 94·4, 93·8 |

The feeds were chosen as being representative of the different cereals and additives used in the manufacture. Deviations from a recovery of 100 per cent. are apparent at levels of 360 and 840 g ton$^{-1}$ of chlorhydroxyquinoline. However, as the procedure described was developed specifically for the assay of feeds containing 600 g ton$^{-1}$, the deviations encountered (+6 per cent. at 360 g ton$^{-1}$ and −7 per cent. at 840 g ton$^{-1}$) were considered acceptable. Satisfactory recoveries of chlorhydroxyquinoline (600 g ton$^{-1}$) were obtained from feeds containing the following additives—

| Additive | | | | | Content, p.p.m. |
|---|---|---|---|---|---|
| Copper .. | .. | .. | .. | .. | 100 |
| Zinc .. | .. | .. | .. | .. | 100 |
| Calcium | .. | .. | .. | .. | 1000 |
| Dimetridazole .. | .. | .. | .. | | 100 |
| Arsanilic acid .. | .. | .. | .. | | 250 |

The procedure described is not suitable for the determination of Halquinol in pre-mixes and concentrates. Simpler and more precise procedures for these determinations that make use of the spectrophotometric determination of the iron(III) chelate of Halquinol in a chloroform - acetone solvent can be used (J. E. Fairbrother and W. F. Heyes, unpublished work).

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# Solvent Extraction of Copper(II) and Zinc(II) with 1,5-Diphenylcarbazone

By HISAHIKO EINAGA and HAJIME ISHII*

(*National Institute for Researches in Inorganic Materials, Niihari-gun, Ibaraki-ken, Japan*)

The extraction characteristics of 1,5-diphenylcarbazone and its complexes with the bivalent metal ions copper(II) and zinc(II) in an isobutyl methyl ketone - water system have been studied and the extraction curves of these metal complexes have also been obtained. The copper(II) complex is extracted from a more acidic solution than is the zinc(II) complex. The extraction equilibria have been examined and the extraction constants determined. The spectral properties of the complexes have also been determined and the application of the reagent to the determination of copper and zinc is suggested.

1,5-DIPHENYLCARBAZONE has not been widely used as an analytical reagent for the separation and determination of trace amounts of metals in spite of the close similarity of its chemical structure to that of dithizone (1,5-diphenylthiocarbazone). It has been thought that 1,5-diphenylcarbazone is "far inferior to dithizone for the purpose."[1] This structural similarity between dithizone and 1,5-diphenylcarbazone, however, implies that the latter possesses considerable potentiality for the formation of complexes with certain of the metal ions that are known to form complexes with the former. Several studies have been made on the reaction between 1,5-diphenylcarbazone and several metal ions, such as iron(II), iron(III), cobalt(II), copper(II), mercury(II), cadmium(II), lead(II) and zinc(II),[2-5] all of which, except iron(III), react with dithizone. It seemed, however, that relatively few details have been reported on the characteristics of solvent extraction of the 1,5-diphenylcarbazone complexes for analytical purposes, which led us to investigate them further. The present paper describes the extraction characteristics of copper(II) and zinc(II) with 1,5-diphenylcarbazone, together with those of the ligand itself, in the solvent system isobutyl methyl ketone - water.

## EXPERIMENTAL

REAGENTS—

*Copper(II) solution*, 1·020 *mg ml⁻¹*—This solution was prepared by dissolving recrystallised copper(II) sulphate pentahydrate in water and acidifying the solution with a small amount of sulphuric acid so as to prevent precipitation of copper(II) by hydrolysis. The solution was standardised by using a complexometric method with 1-(2-pyridylazo)-2-naphthol as a metallochromic indicator.[6]

*Zinc(II) solution*, 1·150 *mg ml⁻¹*—This solution was prepared by dissolving pure zinc metal (99·99 per cent.) in hydrochloric acid, a slight excess of which was added in order to prevent precipitation of zinc(II) by hydrolysis. This solution was also standardised by a complexometric method with Eriochrome black T as a metallochromic indicator.[7]

*1,5-Diphenylcarbazone*, 0·020 *per cent.* (8·32 × 10⁻⁴ M) *solution in isobutyl methyl ketone*—The 1,5-diphenylcarbazone used to prepare this solution was obtained from Kanto Chemical Co., Inc., Japan, and was purified before use by extraction with diethyl ether so as to remove any 1,5-diphenylcarbazide[8] and was finally recrystallised from ethanol.

Adjustment of the pH and ionic strength of the aqueous phase was carried out by using 0·2 M sodium acetate - 0·2 M acetic acid or 0·05 M sodium borate - 0·1 M hydrochloric acid or sodium hydroxide solution for pH adjustment and 1·0 M ammonium chloride solution to adjust the ionic strength.

* Present address: Department of Applied Chemistry, Faculty of Engineering, Tohoku University, Sendai, Japan.

APPARATUS—

Absorption spectra and absorbances at the specified wavelength were obtained with a recording spectrophotometer, Model EPS-3T (Hitachi Ltd., Japan), and a spectrophotometer, Model 139 (Hitachi Ltd., Japan), respectively, with matched 10-mm silica cuvettes. The pH of the equilibrated aqueous phase was measured with a glass electrode - saturated calomel electrode pair and pH meters, Model HM-5A (Toa Dempa Co. Ltd.) and Model F-5 (Horiba Ltd., Japan). A solution $0.09$ M in ammonium chloride and $0.01$ M in hydrochloric acid was defined as $-\log[H^+] = 2.00$. Equilibration of both organic and aqueous phases was carried out with a universal shaker (Model KM, Iwaki Ltd., Japan) at the rate of 300 strokes per minute and at a temperature of 25 °C.
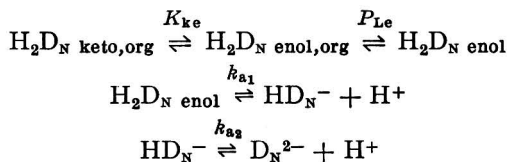
PROCEDURE—

Measurements of extractability (percentage extraction) and distribution ratios of the metal complexes were obtained by the following procedure. A mixture of $n$ ml of the 1,5-diphenyl-carbazone solution and $(20.0 - n)$ ml of isobutyl methyl ketone was equilibrated at various pH values with $20.0$ ml of an aqueous solution that contained a definite amount of metal ions and was adjusted to an ionic strength of $0.10$ M with ammonium chloride. After separation of the phases, the metal ions extracted into the organic phase were determined by measuring absorbances at a specified wavelength and by making use of the molar absorptivity of each metal ion (see under Extraction characteristics of the complexes of copper(II) and zinc(II) with 1,5-diphenylcarbazone). The separated aqueous phase was used for measurement of hydrogen-ion concentration. The concentration of metal ions in the aqueous phase was obtained as the difference between the initial concentration and that in the organic phase, and the distribution ratio, $D_M'$, the ratio of the concentration of the metal ions in the organic phase to that in the aqueous phase, was calculated from these results.

The above procedure was also applied in the absence of metal ions to the determination of the extractability and distribution ratio of the ligand itself. In this instance the concentration of 1,5-diphenylcarbazone in the organic phase was determined by shaking the separated organic phase with an acetate buffer solution (pH 5) and then measuring absorbances at 560 nm (a calibration graph had been constructed by using purified 1,5-diphenylcarbazone). This shaking treatment was necessary in order to obtain reproducible and uniform absorption characteristics of 1,5-diphenylcarbazone, the concentration of which in the aqueous phase was obtained as described previously for the metal ions.

## RESULTS AND DISCUSSION

EXTRACTION CHARACTERISTICS OF 1,5-DIPHENYLCARBAZONE—

Consideration of the structure of 1,5-diphenylcarbazone suggests that its ketonic and enolic forms are present in tautomeric equilibrium in the solution. From its behaviour on neutralisation with sodium hydroxide it has been reported that 1,5-diphenylcarbazone is a monobasic acid.[3] It is, however, reasonable to consider it to be a dibasic acid, just as the structurally closely similar dithizone is a dibasic acid ($k_{a_1}$ = about $2 \times 10^{-5}$ and $k_{a_2} \ll 10^{-15}$).[9] Studies were therefore first made on the spectral and extraction characteristics of 1,5-diphenylcarbazone in an isobutyl methyl ketone - water system, and these characteristics are shown in Figs. 1 and 2, respectively. The absorption spectrum of this reagent did not vary with change in pH of the equilibrated aqueous phase below 8, and had an absorption maximum at 460 nm, which showed, however, a gradual bathochromic shift when the pH was increased and reached a constant value above pH 11 ($\lambda_{max.} = 505$ nm at pH 11·25). The distribution of the reagent from the organic to aqueous phase also became appreciable when the pH was above 8 and gradually increased with increasing pH. The reagent in the aqueous phase had an absorption maximum at 495 nm, which was independent of the pH. These results can be qualitatively interpreted as follows: 1,5-diphenylcarbazone is present in the organic phase in ketonic ($\lambda_{max.} = 460$ nm) and enolic forms ($\lambda_{max.} = 505$ nm) in tautomeric equilibrium, which is, however, gradually shifted in favour of the enolic form by equilibration with an aqueous solution at pH above 8. The enolic form is then distributed into the aqueous phase in which it dissociates into a proton and the anion, $HD_N^-$ ($\lambda_{max.} = 495$ nm), where $H_2D_N$ represents the undissociated 1,5-diphenylcarbazone. A series of these equilibria can be represented as follows—

$$H_2D_N \text{ keto,org} \overset{K_{ke}}{\rightleftharpoons} H_2D_N \text{ enol,org} \overset{P_{Le}}{\rightleftharpoons} H_2D_N \text{ enol}$$

$$H_2D_N \text{ enol} \overset{k_{a1}}{\rightleftharpoons} HD_N^- + H^+$$

$$HD_N^- \overset{k_{a2}}{\rightleftharpoons} D_N^{2-} + H^+$$

where the subscript keto represents the ketonic form, enol the enolic form, org the organic phase and absence of subscript the aqueous phase. These equilibria can be expressed as follows—

$$K_{ke} = [H_2D_N]_{keto,org}/[H_2D_N]_{enol,org} \quad \cdots \quad \cdots \quad \cdots \quad (1)$$

$$P_{Le} = [H_2D_N]_{enol,org}/[H_2D_N]_{enol} \cdots \quad \cdots \quad \cdots \quad \cdots \quad (2)$$

$$k_{a1} = [H^+][HD_N^-]/[H_2D_N]_{enol} \cdots \quad \cdots \quad \cdots \quad \cdots \quad (3)$$

$$k_{a2} = [H^+][D_N^{2-}]/[HD_N^-] \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad (4$$
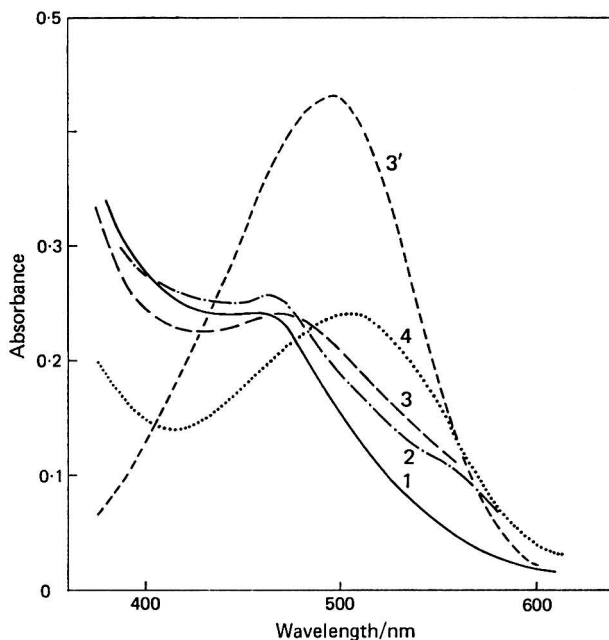


Fig. 1. Absorption characteristics of 1,5-diphenyl-carbazone in the isobutyl methyl ketone - water system. [1,5-Diphenylcarbazone] $4·16 \times 10^{-4}$ M; and [NH$_4$Cl] $0·1$ M. 1 to 4, absorption spectra of organic phase. pH value of equilibrated aqueous phase: 1, 5·25; 2, 8·80; 3, 10·20; and 4, 11·25. 3′, Absorption spectrum of aqueous phase; pH 10·20

Further, let us define $P_L$ as follows—

$$P_L = ([H_2D_N]_{keto,org} + [H_2D_N]_{enol,org})/[H_2D_N]_{enol}$$
$$= [H_2D_N]_{org}/[H_2D_N]_{enol} \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad (5)$$

where $[H_2D_N]_{org}$ is the total concentration of 1,5-diphenylcarbazone in the organic phase. The terms $P_{Le}$ and $P_L$ can be correlated in the equation

$$P_L = (1 + K_{ke})P_{Le} \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad (6)$$

The distribution of 1,5-diphenylcarbazone can then be defined by

$$D_L = \frac{[H_2D_N]_{keto,org} + [H_2D_N]_{enol,org}}{[H_2D_N]_{enol} + [HD_N^-] + [D_N^{2-}]} \qquad .. \qquad .. \qquad .. \qquad (7)$$

where the partition coefficient of the ketonic form was assumed to be so large that its effect in the aqueous phase can be neglected as compared with other species, which assumption is considered to be reasonable having regard to our results. By making use of equations (1) and (6), equation (7) can be rewritten in the following logarithmic form—

$$\text{Log } D_L = \log P_L - \log (1 + k_{a1}/[H^+] + k_{a1}k_{a2}/[H^+]^2) \qquad .. \qquad .. \qquad (8)$$

Equation (8) implies that—

    (i) log $D_L$ should have no dependence on log $[H^+]$ if $H_2D_N$ enol is the principal species in the equilibrated aqueous phase;

    (ii) log $D_L$ should have a linear relationship to log $[H^+]$ with a slope of unity if $HD_N^-$ is the principal species or with a slope of two if $D_N^{2-}$ is the principal species; and

    (iii) there should be a non-linear relationship between log $D_L$ and log $[H^+]$ with a tangential slope between 0 and 1 or 1 and 2, depending on whether appreciable concentrations of both $H_2D_N$ enol and $HD_N^-$ or $HD_N^-$ and $D_N^{2-}$, respectively, are present in the aqueous phase.

Fig. 2 shows the experimental results obtained for log $D_L$ and log $[H^+]$. A linear relationship exists between them with a slope of unity, indicating that $HD_N^-$ is the principal species in the aqueous phase. Equation (8) can therefore be simplified as follows—

$$\text{Log } D_L = \log P_L - \log k_{a1} + \log [H^+] \qquad .. \qquad .. \qquad .. \qquad (8a)$$

The extraction constant of 1,5-diphenylcarbazone, $K_{exL}$, which is expressed by the equilibrium

$$H_2D_N \text{ keto + enol,org} \overset{K_{exL}}{\rightleftharpoons} H^+ + HD_N^-$$

can be defined as follows—

$$K_{exL} = \frac{[H_2D_N]_{keto,org} + [H_2D_N]_{enol,org}}{[HD_N^-][H^+]}$$

$$= P_L/k_{a1} \qquad .. \qquad .. \qquad .. \qquad .. \qquad .. \qquad (9)$$

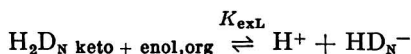The value of $K_{exL}$ was calculated by using the results in Fig. 2 and was determined as log $K_{exL} = 11 \cdot 15$.
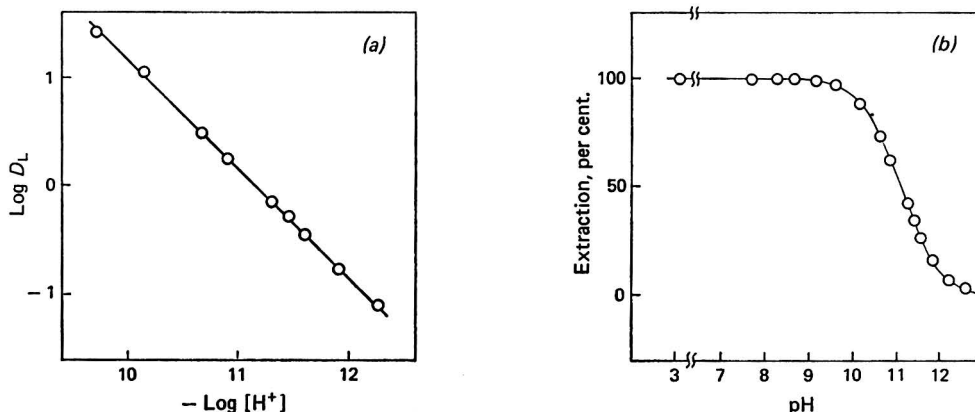


Fig. 2. Extraction characteristics of 1,5-diphenylcarbazone. [1,5-Diphenylcarbazone] $4 \cdot 16 \times 10^{-4}$ M; and [NH$_4$Cl] $0 \cdot 1$ M. (a), Extraction curve of 1,5-diphenylcarbazone; and (b), dependence of distribution ratio on hydrogen-ion concentration

GENERAL TREATMENT OF THE EXTRACTION EQUILIBRIA OF METAL(II) - 1,5-DIPHENYLCARBAZONE COMPLEXES—

The terms used below are defined as follows: $K_{eq}$ is the equilibrium constant of the extraction of the metal complex $M(HN_N)_2(H_2D_N)_{n-2}$, $K_{ex}$ the extraction constant and $P_M$ the

partition coefficient of the same metal complex, $D_M$ the distribution ratio of the metal, $D_M = [M(HD_N)_2(H_2D_N)_{n-2}]_{org}/[M^{2+}]$, and $D_M'$ the apparent distribution ratio of the metal, $D_M' = [M(HD_N)_2(H_2D_N)_{n-2}]_{org}/[M^{2+}]_{total}$ ($[M^{2+}]_{total}$ is the total concentration of the metal in the aqueous phase). $K_{nn'}$ is the equilibrium constant in the aqueous phase of the metal complex $M(D_N)_n H_n'^{2+n'-2n}$, $\beta$ series are formation constants of the side reaction of the metal ion with the species indicated, and $\alpha_{coeff}$ the side-reaction coefficient of the metal ion.

In the extraction equilibria of bivalent metal ions with 1,5-diphenylcarbazone, both ketonic and enolic forms can be considered to participate to an equal extent in the extraction reaction. It is, however, very difficult from equilibration studies, although unimportant in so far as it affects the extraction equilibria, to elucidate which of the forms is the principal participant in the reaction. Therefore, it was considered that both forms of 1,5-diphenyl-carbazone participate in the reaction involvi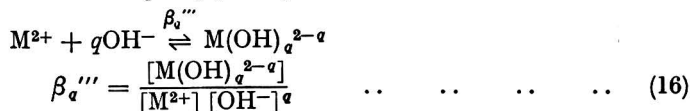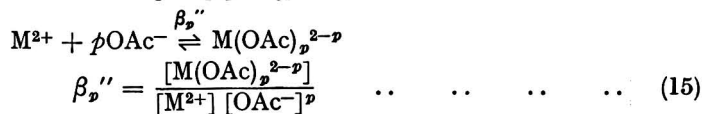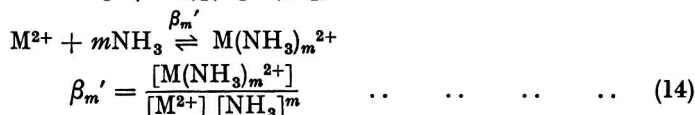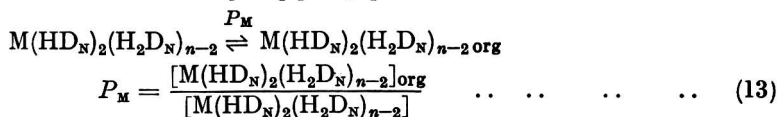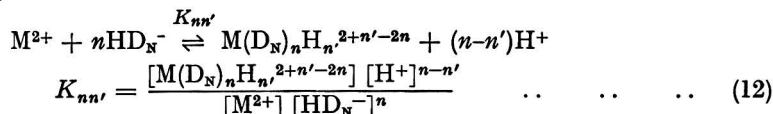ng the extraction of metal complexes. In addition to the assumption made above, it should be considered that the extracted metal complexes are electrically neutral. Under these conditions the principal extraction equilibrium can be represented as follows:

$$M^{2+} + nH_2D_{N\ org} \overset{K_{eq}}{\rightleftharpoons} M(HD_N)_2(H_2D_N)_{n-2\ org} + 2H^+$$

$$K_{eq} = \frac{[M(HD_N)_2(H_2D_N)_{n-2}]_{org}[H^+]^2}{[M^{2+}][H_2D_N]_{org}^n}$$

$$= D_M[H^+]^2[H_2D_N]^{-n} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (10)$$
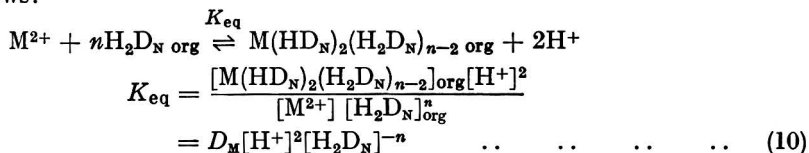
The term $D_M'$, which can easily be obtained experimentally, can be defined as follows—

$$D_M' = \frac{[M(HD_N)_2(H_2D_N)_{n-2}]_{org}}{[M^{2+}] + \sum_1^{n'}\sum_1^{n}[M(D_N)_n H_n'^{2+n'-2n}] + \sum_1^{m}[M(NH_3)_m^{2+}] + \sum_1^{p}[M(OAc)_p^{2-p}] + \sum_1^{q}[M(OH)_q^{2-q}]} \qquad (11)$$

where the following equilibria were taken into consideration—

$$M^{2+} + nHD_N^- \overset{K_{nn'}}{\rightleftharpoons} M(D_N)_n H_n'^{2+n'-2n} + (n-n')H^+$$

$$K_{nn'} = \frac{[M(D_N)_n H_n'^{2+n'-2n}][H^+]^{n-n'}}{[M^{2+}][HD_N^-]^n} \qquad .. \qquad .. \qquad .. \qquad (12)$$

$$M(HD_N)_2(H_2D_N)_{n-2} \overset{P_M}{\rightleftharpoons} M(HD_N)_2(H_2D_N)_{n-2\ org}$$

$$P_M = \frac{[M(HD_N)_2(H_2D_N)_{n-2}]_{org}}{[M(HD_N)_2(H_2D_N)_{n-2}]} \qquad .. \quad .. \qquad .. \qquad .. \qquad (13)$$

$$M^{2+} + mNH_3 \overset{\beta_m'}{\rightleftharpoons} M(NH_3)_m^{2+}$$

$$\beta_m' = \frac{[M(NH_3)_m^{2+}]}{[M^{2+}][NH_3]^m} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (14)$$

$$M^{2+} + pOAc^- \overset{\beta_p''}{\rightleftharpoons} M(OAc)_p^{2-p}$$

$$\beta_p'' = \frac{[M(OAc)_p^{2-p}]}{[M^{2+}][OAc^-]^p} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (15)$$

$$M^{2+} + qOH^- \overset{\beta_q'''}{\rightleftharpoons} M(OH)_q^{2-q}$$

$$\beta_q''' = \frac{[M(OH)_q^{2-q}]}{[M^{2+}][OH^-]^q} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (16)$$

Rearrangement of equation (11) by using equations (10) and (12) to (16) gives—

$$\frac{1}{D_M'} = \frac{1}{P_M} + \frac{[H^+]^2[H_2D_N]_{org}^{-n} \times \alpha_{coeff}}{K_{eq}}$$

$$\alpha_{coeff} = 1 + \sum_1^{n'}\sum_1^{n} K_{nn'}[HD_N^-]^{-n}[H^+]^{n-n'} + \sum_1^{m} \beta_m'[NH_3]^m$$

$$+ \sum_1^{p} \beta_p''[OAc^-]^p + \sum_1^{q} \beta_q'''[OH^-]^q .. \qquad .. \qquad .. \qquad (17)$$

Further, it should be appropriate to assume that the presence in the aqueous phase of lower and higher order complexes for the 1,5-diphenylcarbazone ligand than the electrically neutral complex $M(HD_N)_2(H_2D_N)_{n-2}$ can be neglected and that the neutral complex has a partition coefficient that is high enough to be able to make $1/P_M \ll 1$. With these assumptions, equation (17) can be further simplified into logarithmic form—

$$\text{Log } D_M' + \log \alpha_{coeff} = \log K_{eq} - 2 \log [H^+] + n \log [H_2D_N]_{org}$$

$$\alpha_{coeff} = 1 + \sum_1^m \beta_m'[NH_3]^m + \sum_1^p \beta_p''[OAc^-]^p + \sum_1^q \beta_q'''[OH^-]^q \quad .. \quad (18)$$

Equation (18) implies that a linear relationship should exist between $\log D_M' + \log \alpha_{coeff}$ and $\log [H^+]$ under constant conditions of $\log [H_2D_N]_{org}$ with a slope of 2 and $\log D_M' + \log \alpha_{coeff}$ and $\log [H_2D_N]_{org}$ under constant conditions of $\log [H^+]$ with a slope of $n$, which corresponds to the actual number of ligands in the extracted complex.

Now let us define the term extraction constant, $K_{ex}$, as follows:

$$M^{2+} + n\,HD_N^- \overset{K_{ex}}{\rightleftharpoons} M(HD_N)_2(H_2D_N)_{n-2\ org} + (2-n)\,H^+$$

$$K_{ex} = \frac{[M(HD_N)_2(H_2D_N)_{n-2}]_{org}[H^+]^{2-n}}{[M^{2+}][HD_N^-]^n}$$

$$= P_M K_{n\,2(n-1)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (19)$$

The following relationship can be obtained from equations (2), (3), (10) and (19)—

$$\text{Log } K_{ex} = \log K_{eq} + n \log K_{exL} \quad .. \quad .. \quad .. \quad (20)$$

The term $K_{ex}$ indicates how easily the complex can be formed in the aqueous phase and extracted into the organic phase, and can be obtained by using the value of $K_{exL}$ as determined in the preceding section.

EXTRACTION CHARACTERISTICS OF THE COMPLEXES OF COPPER(II) AND ZINC(II) WITH 1,5-DI-
PHENYLCARBAZONE—

Preliminary experiments have shown that zinc(II) cannot be extracted as its 1,5-diphenylcarbazone complex into the organic phase when ammonium salts are absent, although the extraction of the copper(II) complex was not influenced by the presence or absence of ammonium salts. This difference in behaviour may be due to the formation of hydroxo species of zinc(II), especially the zincate ion, which retards or inhibits the formation of the zinc(II) complex. It is therefore necessary to add ammonium salt to the aqueous phase



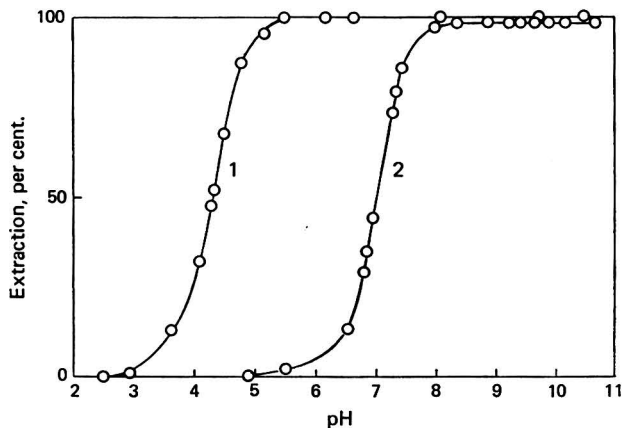Fig. 3. Extraction curves of copper(II) and zinc(II) as 1,5-diphenylcarbazone complexes. 1, Extraction curve of copper(II): [copper(II)] $8 \cdot 03 \times 10^{-6}$ M; [1,5-diphenylcarbazone] $2 \cdot 08 \times 10^{-4}$ M; and [NH$_4$Cl] $0 \cdot 1$ M. 2, Extraction curve of zinc(II): [zinc(II)] $1 \cdot 76 \times 10^{-5}$ M; [1,5-diphenylcarbazone] $4 \cdot 16 \times 10^{-4}$ M; and [NH$_4$Cl] $0 \cdot 1$ M

and thus convert the zinc(II) into the more labile ammine complexes. The presence of ammonium chloride at a concentration of 0·1 M was concluded to be sufficient for the extraction of zinc(II) as its 1,5-diphenylcarbazone complex.
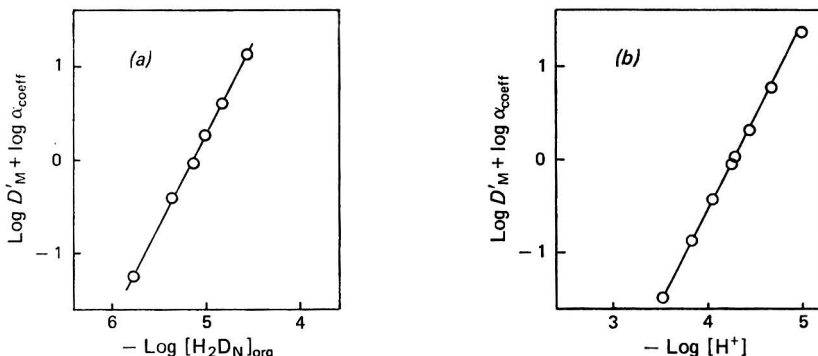


Fig. 4. Extraction characteristics of copper(II) - 1,5-diphenylcarbazone complex. [Copper(II)] $8·03 \times 10^{-6}$ M; and [NH$_4$Cl] 0·1 M. (a), $-$Log [H$^+$] 6·12; and (b), $-$log [H$_2$D$_N$]$_{org}$ 3·70

Extraction curves for the copper(II) and zinc(II) complexes are presented in Fig. 3, which show that copper(II) can be extracted into the organic phase from more acidic solution (pH$_{1/2}$ = 4·30) than is zinc(II) (pH$_{1/2}$ = 7·02). Under the conditions specified in Fig. 3, copper(II) was extracted quantitatively (more than 99 per cent.) and zinc(II) almost quantitatively (98 per cent.) by a single extraction. It is therefore necessary to carry out a second extraction for the quantitative extraction of zinc(II).
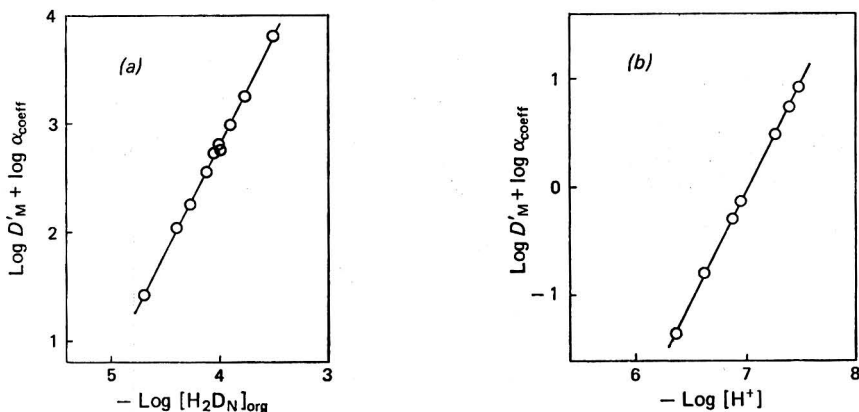


Fig. 5. Extraction characteristics of zinc(II) - 1,5-diphenylcarbazone complex. [Zinc(II)] $1·76 \times 10^{-5}$ M; and [NH$_4$Cl] 0·1 M. (a), $-$Log [H$^+$] 8·90; and (b), $-$log [H$_2$D$_N$]$_{org}$ 3·40

The extraction characteristics of copper(II) and zinc(II) complexes are presented in Figs. 4 and 5, respectively; in each instance $\alpha_{coeff}$ was calculated by using the formation constants summarised in Table I with suitable modification for some of the results due to the change in ionic strength. It is evident from Figs. 4 and 5 that linear relationships exist for both copper(II) and zinc(II) between log $D_M'$ + log $\alpha_{coeff}$ and log [H$^+$], the slope of which is 2, as expected from equation (18). Linear relationships also occur for both metal ions between log $D_M'$ + log $\alpha_{coeff}$ and log [H$_2$D$_N$]$_{org}$ with a slope of 2 ($n = 2$). Therefore, the extraction equilibrium [equation (10)] and extraction constant as defined in equation (19) can be simplified as follows—

$$M^{2+} + 2H_2D_{N\ org} \overset{K_{eq}}{\rightleftharpoons} M(HD_N)_{2\ org} + 2H^+$$
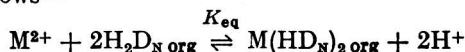
$$K_{eq} = \frac{[M(HD_N)_2]_{org}[H^+]^2}{[M^{2+}][H_2D_N]_{org}^2} \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad (10a)$$

$$M^{2+} + 2HD_N^- \overset{K_{ex}}{\rightleftharpoons} M(HD_N)_{2\,org}$$
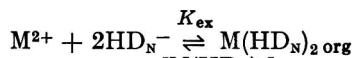
$$K_{ex} = \frac{[M(HD_N)_2]_{org}}{[M^{2+}][HD_N^-]^2}$$

$$= P_M K_{22} \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad (19a)$$

and

$$Log\,K_{ex} = \log K_{eq} + 2\log K_{exL} \quad \cdots \quad \cdots \quad \cdots \quad (20a)$$

Values of $K_{eq}$ for both copper(II) and zinc(II) were calculated from the results presented in Figs. 4 and 5, respectively, and they are summarised in Table II. Values of $K_{ex}$ were also calculated from the values of $K_{eq}$ and $K_{exL}$, and they are also summarised in Table II.

TABLE I

FORMATION CONSTANTS USED FOR THE CALCULATION OF $\alpha_{coeff}$[10]

| Cation | Term | Ligand | | |
|---|---|---|---|---|
| | | OAc$^-$ | NH$_3$ | OH$^-$ |
| H$^+$ | p$k_a$ | 4·65 | 9·37 | 13·80 |
| Copper(II) | Log $\beta_1$ | 1·7 | 4·13 | 6·0 |
| | Log $\beta_2$ | 2·7 | 7·61 | |
| | Log $\beta_3$ | 3·1 | 10·48 | Log $K_{Cu_2(OH)_2}^{2OH} = 17·1$ |
| | Log $\beta_4$ | 2·9 | 12·59 | |
| Zinc(II) | Log $\beta_1$ | 1·3 | 2·27 | 4·4 |
| | Log $\beta_2$ | 2·1 | 4·61 | — |
| | Log $\beta_3$ | — | 7·01 | 14·4 |
| | Log $\beta_4$ | — | 9·06 | 15·5 |

Results were for $\mu = 0\cdot1$ except with the hydroxo complexes of copper(II) and zinc(II), which were for $\mu = 0$ and were converted into $\mu = 0\cdot1$ in the usual manner when calculations of $\alpha_{coeff}$ were carried out. For acid dissociation of ligands, the acid-dissociation constant, p$k_a$, was cited instead of the formation constant.

Copper(II) and zinc(II) complexes with 1,5-diphenylcarbazone [Cu(HD$_N$)$_2$ and Zn(HD$_N$)$_2$, respectively] thus extracted into isobutyl methyl ketone have their absorption maxima at 530 and 520 nm, respectively, and the very large values of molar absorptivities of $7\cdot6 \times 10^4$ (530 nm) for the copper(II) complex and $5\cdot8 \times 10^4$ (520 nm) for the zinc(II) complex suggest that the extraction into isobutyl methyl ketone of trace amounts of copper and zinc as their 1,5-diphenylcarbazone complexes can be utilised for their spectrophotometric determination.

TABLE II

EXTRACTION CHARACTERISTICS OF COPPER(II) AND ZINC(II)

| Metal | Composition of complex | Absorption maximum, $\lambda_{max.}$/nm | Molar absorptivity, $\epsilon_{max.}$ | Log $K_{eq}$ | Log $K_{ex}$ |
|---|---|---|---|---|---|
| Cu | Cu(HD$_N$)$_2$ | 530 | $7\cdot6 \times 10^4$ | $-1\cdot11$*<br>$-1\cdot10$† } | 21·2 |
| Zn | Zn(HD$_N$)$_2$ | 520 | $5\cdot8 \times 10^4$ | $-7\cdot10$*<br>$-7\cdot13$† } | 15·2 |
| | | | | Log $K_{exL} = 11\cdot15.$ | |

* Results obtained from the relationship between $\log D_M' + \log \alpha_{coeff}$ and $\log [H_2D_N]_{org}$.
† Results obtained from the relationship between $\log D_M' + \log \alpha_{coeff}$ and $\log [H^+]$.

Balt and van Dalen[5] reported that copper(II) and zinc(II) complexes with 1,5-diphenyl-carbazone have the following characteristics: $\mu = 0.1$ (NaClO$_4$) at 20 to 22 °C; log $K_{eq} = 1.27$ for copper(II) and $-6.76$ for zinc(II); $\lambda_{max}$, 530 nm for copper(II) and zinc(II); and $\epsilon_{\lambda max}$, $6.8 \times 10^4$ for copper(II) and $3.7 \times 10^4$ for zinc(II), which were determined by using a toluene - water system. By using these results for log $K_{eq}$, and partition coefficient ($P_L = 39$) and acid-dissociation constant ($k_{a1} = 2.9 \times 10^{-9}$),[5] the values of log $K_{ex}$ were calculated according to equations (9) and (20a) to be 21.5 for copper(II) and 13.5 for zinc(II). Fairly good agreement can be seen between the results found by Balt and van Dalen[5] and those obtained in the present work for log $K_{ex}$, although the partition systems used were different in the two instances. Some differences in these results, especially in the value for log $K_{ex}$ for zinc(II), and also in other characteristics (for example, molar absorptivity) might be partly due to the differences in the nature of the extraction systems toluene - water and isobutyl methyl ketone - water, which should, however, be elucidated in future from the standpoint of solution theory.

The copper(II) and zinc(II) complexes showed no photochemical change under ordinary laboratory lighting conditions and are considered to be stable unless exposed to intense ultraviolet radiation.

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# A Solvent-saving Extraction - Evaporation Apparatus Developed for Residue Analysis of Pesticides

By G. VOSS and W. BLASS

(*Agrochemical Division, CIBA-Geigy Ltd., Basle, Switzerland*)

An apparatus that simultaneously allows Soxhlet extraction of pesticides and concentration of the resulting extract has been designed. The evaporated solvent is refluxed into the Soxhlet flask where it is utilised again for the extraction. The major advantages of this cyclic extraction - evaporation system are that redistillation of solvents prior to extraction can be omitted, that manual work is reduced, as the solvent evaporation process occurs simultaneously with the extraction of the sample, and that excess of solvent is not wasted but re-utilised for extraction, which is of interest from the economic and environmental contamination points of view.

A NUMBER of organic solvents have been used for extracting pesticides from various substrates. Owing to the accuracy of detection required for this type of compound, and also for technical reasons, the initial extracts must be concentrated to a relatively small volume by evaporation before chromatographic clean-up procedures can be applied. The evaporated portion of the solvent is lost for any further work and also contributes to air and water pollution. In order to avoid, or at least reduce, this economic and environmental drawback, an apparatus was designed that permits the re-utilisation of evaporated solvents without complicating the extraction procedure. Because exhaustive Soxhlet extraction has recently been recommended for the analysis of all organophosphorus insecticides,[1] and as this procedure is well known to residue chemists, we have modified the Soxhlet apparatus as described below.
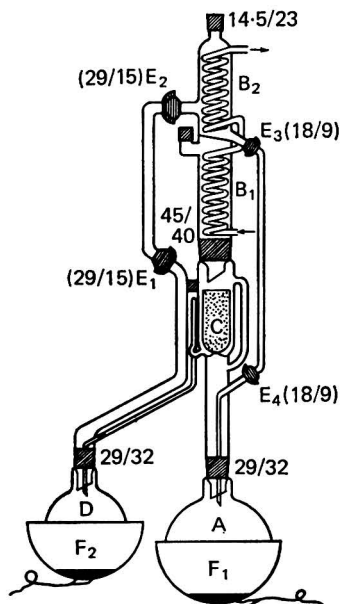


Fig. 1. Extraction - evaporation apparatus designed for residue analysis of pesticides

APPARATUS—

Details of the apparatus are shown in Fig. 1. The solvent (400 to 500 ml), present in a 1-litre round-bottomed flask (A), is heated and refluxed from the lower part of the condenser ($B_1$) into the Soxhlet thimble (C). From the central part of the Soxhlet apparatus the solvent is siphoned into a 250-ml round-bottomed flask (D), from which it is continuously distilled into the upper part of the water-cooled condenser ($B_2$) through a detachable glass tube joined at two ball and socket joints ($E_1$ and $E_2$). The organic solvent is condensed in $B_2$ and refluxed through a second glass tube (equipped with ball joints $E_3$ and $E_4$) back into flask A. The adjustment of the temperatures in the heating jackets ($F_1$ and $F_2$) depends on the solvent used for extraction and is easily determined by experiment. The rates of distillation that occur in flasks A and D should be similar, thus avoiding both evaporation to complete dryness and overflow in flask D.

RESULTS AND DISCUSSION

Duplicate recovery experiments were carried out with a number of pesticides that differed in their chemical and physicochemical properties, and with various substrates (plant materials, meat and soil) and solvents. The present extraction - evaporation procedure was found to result in satisfactory recovery values, as summarised in Table I.

TABLE I

RECOVERIES OBTAINED WITH DIFFERENT MATERIALS AND PESTICIDES
BY USING THE EXTRACTION - EVAPORATION APPARATUS

| Pesticide | Material | Solvent | Deter-mination* | Added, p.p.m. | Recovered, per cent. |
|---|---|---|---|---|---|
| Atrazine | Sandy loam | Methanol | (i) | 1·0 | 100,† 75‡ |
| Chlorobenzilate | Apples | Acetone | (ii) | 0·5 | 77, 87§ |
| DDT | Meat | n-Hexane | (ii) | 0·5 | 85, 96‡ |
| Dichlorvos | Wheat grain | Methylene chloride | (iii) | 5·0 | 100, 110† |
| Methidathion | Orange peel | Chloroform - methanol (9 + 1) | (i) | 2·0 | 88, 92‡ |
| Monocrotophos | Cotton seeds | Chloroform - methanol (9 + 1) | (iv) | 0·2 | 74, 75¶ |

* (i), Gas - liquid chromatography with flame-ionisation detection; (ii), gas - liquid chromatography with electron-capture detection; (iii), cholinesterase inhibition; and (iv), gas - liquid chromatography with flame-photometric detection.
 † No clean-up.
 ‡ Column chromatographic clean-up.
 § Thin-layer chromatographic clean-up.
 ¶ Partitioning, column and thin-layer chromatographic clean-up.

The major advantages of using this apparatus are as follows. Firstly, because those distilled solvent portions which actually extract the analytical material in the thimble are siphoned into flask D (Fig. 1) instead of flowing back into flask A, the often necessary re-distillation of solvents in glass prior to extraction of samples can be omitted. This advantage saves a considerable amount of manpower.

Secondly, at the end of a 3 to 4-hour extraction period the final extract can be recovered from flask D in a relatively concentrated form. Again, this saves time and manpower, even if further evaporation for final volume adjustment proves to be necessary.

Thirdly, losses of solvents are minimised. The initial amount of solvent placed in flask A can be used for many subsequent extractions, at least within a series of the same type of pesticide-containing sample. The advantage is of particular importance with expensive solvents.

Fourthly, in most laboratories organic solvents are evaporated by streams of air, Kuderna-Danish evaporators, or rotating evaporators attached to water pumps, thus contributing to the contamination of air and water. This disadvantage is avoided by the process of recycling the solvents in the system between flasks D and A. In our experience the apparatus can be operated in the open laboratory and a fume hood is not required.

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# Fractionation and Identification of Commercial Hydrocolloid Stabilising Agents

## Part II.* Identification of the Components of Guar Gum - Locust Bean Gum and of Pectinate - Gum Tragacanth Mixtures

By R. G. MORLEY, G. O. PHILLIPS, D. M. POWER

(*Department of Chemistry and Applied Chemistry, University of Salford, Salford, Lancashire, M5 4WT*)

AND R. E. MORGAN

(*The Medical Center, University of Alabama, Birmingham, Alabama, U ...A.*)

The limitations of a method for the identification of mixtures of stabilising agents described previously have been overcome. The method now facilitates positive identification of pectinate and tragacanth in admixture and of guar gum and locust bean gum in admixture.

A METHOD for the fractionation and identification of the components of stabiliser mixtures has been described previously.[1,2] The method suffers from two limitations, namely that guar gum and locust bean gum cannot be positively identified when they occur in admixture, and that the identification of pectinate and tragacanth in admixture requires experience in assessing the extent to which the precipitate redissolves on addition of excess of iron(III) chloride. These limitations have now been overcome.

## EXPERIMENTAL

The detailed experimental procedure has been described previously.[1]

### PECTINATE - GUM TRAGACANTH FRACTIONATION—

The clear liquid retained after precipitation with 1 N hydrochloric acid in the original procedure[1] is neutralised with sodium hydroxide solution (but not made alkaline). Ethanol (3 volumes) is then added and the precipitate that forms is redissolved in water to give a 0·5 per cent. solution, which is divided into three portions. Two portions are treated as described previously.[1] The third portion (1 volume) is added to 2 M potassium hydroxide solution (1 volume) and the solution centrifuged. Formation of a precipitate indicates the presence of pectinate. The supernatant liquid is then made just acidic to litmus by the dropwise addition of glacial acetic acid. Lead acetate solution ($\frac{1}{5}$ volume) is added and the white, curdy precipitate formed is dispersed by the dropwise addition of glacial acetic acid. The presence of a gel on centrifugation indicates the presence of gum tragacanth.

### GUAR GUM AND LOCUST BEAN GUM FRACTIONATION—

Amberlite IR-45 resin (exchange capacity 2 mequiv ml$^{-1}$ of wet resin) is regenerated in the hydroxide form by stirring it with 4 per cent. sodium hydroxide solution for 1 hour. The resin is then washed free from excess of sodium hydroxide, stirred with 5 per cent. boric acid solution for 30 minutes and packed in a 30 × 2-cm column. The column is washed with boric acid solution and the excess of borate finally removed with water. Finally, 250 ml of 4 per cent. cetylpyridinium chloride solution is percolated through the column and the eluate is lyophilised to give cetylpyridinium borate.

The clear liquid from the 1 per cent. cetylpyridinium chloride precipitation step in the original method,[1] containing the neutral stabilisers, is divided into three portions. Two portions are treated as described in the original procedure. To the third portion (1 volume) acetic acid is added so that on addition of cetylpyridinium borate (1 volume) a pH of 4·5 is obtained. Sodium hydroxide solution (10$^{-1}$ M) is then added until a pH of 8 is attained. It is important to monitor the pH continuously during these operations and to stir the solution.

* For details of Part I of this series, see reference list, p. 815.

Stock solution (agar 1, alginate 2, arabic 3, carboxymethylcellulose 4, carrageenan 5, guar gum 6, locust bean gum 7, methylcellulose 8, pectate 9, pectinate 10, tragacanth 11).
Add 1 volume 1% cetyl pyridinium chloride; allow to clarify; centrifuge

Precipitate (5).
CONFIRM CARRAGEENAN

Supernatant (1,2,3,4,9,10,11).
Add 3 volumes ethanol; centrifuge

Precipitate (1,2,3,4,9,10,11).
Wash with 80% ethanol; re-dissolve in 1 volume H₂O;
add 1 volume 1N HCl; centrifuge

Supernatant (1,3,10,11).
Adjust to just pH 7; add
3 volumes ethanol; centrifuge

Precipitate (1,3,10,11).
Re-dissolve in H₂O; divide into three portions

1st portion.
Add 1M CuSO₄

Precipitate.
CONFIRMS
PECTINATE

Supernatant.
CONFIRM
ARABIC

2nd portion.
Add FeCl₃

Precipitate.
INDICATES
PECTINATE
AND/OR
TRAGACANTH

Supernatant.
CONFIRM
AGAR

3rd portion.
Add 2M KOH

Precipitate.
CONFIRMS
PECTINATE

Supernatant.
CONFIRM
TRAGACANTH

Precipitate (2,4,9).
Re-dissolve in dilute
NaOH to pH 7; add 1/5
volume trichloroacetic acid; centrifuge

Supernatant
(discard)

Supernatant (6,7,8).
Add 3 volumes ethanol; centrifuge

Precipitate (6,7).
Dissolve in minimum volume H₂O;
divide into two portions

1st portion.
CONFIRM GUAR GUM
OR LOCUST BEAN GUM

2nd portion.
Add 1 volume cetylpyridinium borate
and acetic acid to pH 4.5;
titrate with
0.1M NaOH to pH 8

Precipitate.
CONFIRMS GUAR GUM

Supernatant.
Titrate to pH 8.4

Precipitate.
CONFIRMS LOCUST BEAN GUM

Supernatant (8).
CONFIRM METHYL-
CELLULOSE

Supernatant
(discard)

Supernatant (4).
Adjust to just
pH 7; add 3 volumes
ethanol; centrifuge

Precipitate.
Dissolve in H₂O.
CONFIRM
CARBOXYMETHYLCELLULOSE

Precipitate (2,9).
Re-dissolve in
dilute NaOH just to pH 7;
divide into two portions

1st portion.
CONFIRM
PECTATE

2nd portion.
CONFIRM
ALGINATE

Supernatant
(discard)

Fig. 1.    The fractionation scheme

The formation of a precipitate indicates the presence of guar gum.  The precipitate is removed by centrifugation and the addition of sodium hydroxide solution is continued until a pH of 8·4 is attained.  The formation of a further precipitate at this pH indicates the presence of locust bean gum.  The guar gum - cetylpyridinium borate precipitate is yellow whereas the locust bean gum - cetylpyridinium borate precipitate is white.

The new fractionation scheme, modified on the basis of the results presented here, is shown in Fig. 1.

## DISCUSSION

The fractionation scheme described (Fig. 1) facilitates the separation and identification of gum tragacanth in admixture.  Moreover, the procedure allows guar gum and locust bean gum to be separated readily and identified when they occur in admixture.  The procedure outlined in Fig. 1 represents the first successful chemical separation of these hydrocolloids. The precise pH at which guar and locust bean gum precipitate varies slightly with the nature of the sample.  Thus, solutions of different samples of guar gum have been found to precipitate between pH 7·7 and 8·3; solutions of locust bean gum alone precipitate in the pH range 8·6 to 9·3.  When both hydrocolloids are present in the solution, guar gum precipitates in the pH range 7·8 to 8·3 and locust bean gum in the pH range 9·2 to 9·4.  However, it is important to stress that in every instance, with all samples tested, a clear separation of guar and locust bean gum was achieved.

The analysis scheme (Fig. 1) is still subject to certain minor criticisms, because although $\kappa$-carrageenan samples give a positive methylene blue test, $\lambda$- and $\mu$-carrageenan fractions do not.  In practice, however, this observation does not constitute a difficulty as all commercial samples of carrageenan so far encountered do contain $\kappa$-carrageenan and will accordingly form a blue - black fibrous precipitate in the methylene blue confirmatory test for carrageenan.

The confirmatory test for agar described in the original paper[1] depends on the presence of the anionic fraction.  Industrial samples of agar normally contain this anionic fraction and consequently show a positive reaction.

The fractionation scheme has been successfully used for the analysis of stabiliser mixtures isolated from food products, particularly frozen desserts.

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     NOTE—Reference 1 is to Part I of this series.

# Determination of Nicotinamide in Some Injections of B-complex Vitamins by Thin-layer Chromatography

By SAAD A. ISMAIEL AND DAWOUD A. YASSA

(*Research Department, Société Misr pour l'Industrie Pharmaceutique, 92 El-Mataria Street, Post El-Zeitoun, Cairo, Egypt*)

Nicotinamide in some injections of B-complex vitamins can be separated on a thin layer of silica gel G by using a solvent mixture of ethanol (98 per cent.) - chloroform ($25 + 60 \ V/V$), eluted from the scraped off area with 0·1 N hydrochloric acid and the solution measured spectrophotometrically at 261 nm. Results obtained by use of the proposed method are compared with those obtained with the ammonia distillation method of the British Pharmacopoeia 1968.

NICOTINAMIDE in injection solutions of B-complex vitamins alone, or in the presence of liver extract, cannot be determined by direct measurement of the light absorption in 0·1 N hydrochloric acid[1] because of the interference of the other B-complex vitamins and liver extract, while use of the ammonia distillation method of the British Pharmacopoeia[2] gives high results owing to the interference of the volatile titratable bases formed by the action of sodium hydroxide on the other constituents of the injection during the distillation.

If the cyanogen bromide method of the United States Pharmacopoeia[3] is used, nicotinic acid, when present, will interfere.

Trials with the thin-layer chromatographic method of Sunshine[4] to separate nicotinamide from the other constituents of the injection resulted in incomplete separation of nicotinamide from riboflavine. The thin-layer chromatographic method of Ismaiel and Yassa[5] successfully separated nicotinamide from riboflavine; however, $R_F$ values were found to be 0·6 and 0·46, respectively, thus making the careful scraping of the area containing nicotinamide alone difficult.

In this paper a method is proposed that depends on the thin-layer chromatographic separation of nicotinamide from the other constituents of the injection solution with use of a solvent mixture of ethanol (98 per cent.) - chloroform ($25 + 60 \ V/V$), followed by elution with 0·1 N hydrochloric acid and measurement of the absorption at 261 nm. Use is made of the $E_{1cm}^{1\%}$ value reported by Clarke and Berle.[1] The proposed method can be used to determine nicotinamide in the presence of other B-complex vitamins, liver extract and nicotinic acid.

## EXPERIMENTAL

MATERIALS—

*Silica gel G plates*—These plates are the same as those used in the method of Ismaiel and Yassa.[5]

*Solvent mixture*—The mixture is ethanol (98 per cent.) - chloroform ($25 + 60 \ V/V$). It is preferable to use freshly distilled solvents.

*Hydrochloric acid*, 0·1 N.

SAMPLES—

The formulations of the samples under test are shown in Table I.

PROCEDURE—

Apply, by means of a tuberculin syringe in portions of 0·2 to 0·5 ml, the injection solutions (containing from 15 to 40 mg of nicotinamide, as stated on the label) to the plate as a transverse line about 6 cm long, evaporating the solvent after each application by using a hot stream of air; allow the plate to cool to room temperature. Separate the nicotinamide from the other constituents by allowing the solvent front to travel at least 12 cm, then remove the plate and dry it in air. Scrape off the silica gel G containing the nicotinamide (3 mm from

## TABLE I

### COMPOSITION OF SAMPLES

| | Sample | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Constituent | I | II | III | IV | V | VI | VII | VIII | IX |
| Thiamine hydrochloride/mg | 100 | 100 | 100 | 100 | 100 | 100 | 250 | 100 | 50 |
| Riboflavine/mg .. .. .. | 2 | — | 2 | 1 | 2 | 1 | 5 | 1 | 0·5 |
| Riboflavine-5-phosphate (sodium salt)/mg .. .. | — | 2 | — | — | — | — | — | — | — |
| Nicotinamide/mg .. .. | 200 | 200 | 200 | 100 | 160 | 50 | 30 | 100 | 50 |
| Nicotinic acid/mg .. | — | — | — | — | 40 | — | — | 10 | 5 |
| Pyridoxine hydrochloride/mg .. | 10 | 10 | 10 | 10 | 5 | 10 | 50 | 5 | 10 |
| Calcium pantothenate/mg .. | 1 | 1 | 5 | — | 5 | — | — | — | 5 |
| Sodium pantothenate/mg .. | — | — | — | 7 | — | — | — | 5 | — |
| Cyanocobalamin/μg .. .. | — | — | 100 | 100 | — | — | — | — | 100 |
| Biotin/μg .. .. .. | — | — | — | — | 5 | — | — | 5 | — |
| Benzyl alcohol/mg .. .. | 20 | 20 | 20 | 20 | 20 | 20 | 140 | 20 | 20 |
| Chlorocresol(4-chloro-3-methylphenol)/mg .. .. | 2·5 | 2·5 | 2·5 | 2·5 | 2·5 | 2·5 | — | 2·5 | 2·5 |
| Dry liver extract for parenteral use/mg .. .. .. .. | — | 100 | — | 10 | — | — | — | — | — |
| Water for injection .. .. | to 1 ml | to 1 ml | — | to 1 ml | to 1 ml | to 1 ml | to 1 ml | to 1 ml | to 1 ml |
| Liver injection crude (U.S.P. XV) | — | — | to 1 ml | — | — | — | — | — | — |

the margin of the riboflavine spot to 1 cm away from the solvent front) and transfer it to a 100-ml, wide-mouthed, calibrated flask. Add about 50 ml of 0·1 N hydrochloric acid, shake the flask for 2 minutes, then dilute to volume with 0·1 N hydrochloric acid and centrifuge (or filter through a dry Whatman No. 1 filter-paper, rejecting the first 10-ml portion of the filtrate). Transfer a volume containing about 0·75 to 1·2 mg of nicotinamide to a 100-ml calibrated flask and dilute to the mark with 0·1 N hydrochloric acid. Finally, measure the extinction at 261 nm by using 0·1 N hydrochloric acid as a blank.

For calculation purposes use 454 as the value of $E_{1\,cm}^{1\%}$. The results obtained are given in Table II.

## TABLE II

### COMPARISON BETWEEN THE RESULTS BY THE PROPOSED METHOD AND THE AMMONIA DISTILLATION METHOD

| | Amount of nicotinamide/mg ml$^{-1}$ | | |
|---|---|---|---|
| | Stated | Found by | |
| Sample* | | proposed method† | ammonia distillation method‡ |
| I .. .. .. | 200 | 196·4 (±1·22%) | 218·88 |
| II .. .. | 200 | 196·1 (±1·92%) | 224·5 |
| III .. .. .. | 200 | 194·2 (±1·35%) | 220 |
| IV .. .. .. | 100 | 99·2 (±1·4%) | 112·94 |
| V .. .. .. | 160 | 157·6 (±1·62%) | 173·5 |
| VI .. .. .. | 50 | 50·9 (±1·43%) | 53·7 |
| VII.. .. .. | 30 | 30·8 (±2·12%) | 32·9 |
| VIII .. .. | 100 | 101·2 (±1·8%) | 109·8 |
| IX .. .. .. | 50 | 48·9 (±1·62%) | 54·1 |

* Authentic sample.
† Mean of 6 experiments; values in parentheses are the mean standard deviations of individual results.
‡ Slightly high results due to the interference of volatile titratable bases formed by the action of sodium hydroxide on the other constituents of the injection during the distillation.

The separation of nicotinamide from the other ingredients of the injection was attempted by use of mixtures of ethanol (95 and 98 per cent.) - chloroform - water and ethanol (95 and 98 per cent.) - chloroform in different proportions, but the mixture ethanol (98 per cent.) - chloroform (25 + 60 $V/V$) gave the best separation. It is interesting to note that higher proportions of chloroform than that used in the method separated riboflavine into two spots,
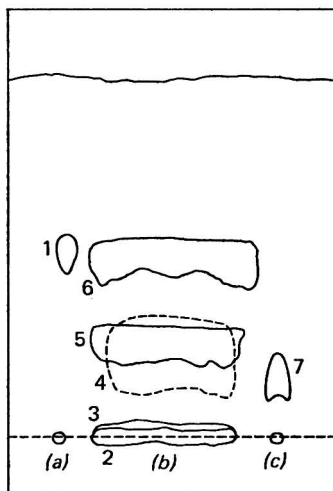
Fig. 1. Developed chromato-gram showing the separation of nicotinamide from the other B-complex vitamins contained in the injection after treatment with cyanogen bromide and aniline. (a) Nicotinamide reference stand-ard; (b) sample V; and (c) nicotinic acid reference standard. Spots 1 and 6 represent nicotinamide, spots 4 and 7 nicotinic acid, spot 5 ribo-flavine, spot 2 thiamine and spot 3 pyridoxine

with tailing from the upper spot reaching the lower one on the starting line. This effect occurs because of a decrease in the solubility of riboflavine on increasing the proportion of chloroform.

The $R_F$ values of the spots on the developed chromatogram, as shown in Fig. 1, are thiamine 0·00, pyridoxine 0·015, riboflavine 0·26, nicotinamide 0·51 and nicotinic acid 0·23. It is also shown that the $R_F$ value of nicotinic acid is affected by the presence of the other vitamins, especially nicotinamide.    Liver extract, if present, however, will give three brown spots, with $R_F$ values of 0·00, 0·02 and 0·98.

The nicotinamide spot can be made visible by leaving the developed chromatogram in a closed jar containing two small beakers, the first of which contains cyanogen bromide solution and the second aniline, deep orange spots representing nicotinamide or nicotinic acid being obtained within 1 hour.    This method was used to determine the boundary of the area containing nicotinamide to be scraped off for the determination.

The absorption spectrum of the measured solution showed that nicotinamide was separated with a purity sufficient for accurate measurement at 261 nm.

Recoveries of the products studied ranged from 94·6 to 103·6 per cent., and the standard deviation ranged from ±1·22 to ±2·12 per cent. ($n = 6$).

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# Determination of Balsamic Acids and Esters by Gas-Liquid Chromatography

By K. J. HARKISS and P. A. LINLEY

(*Postgraduate School of Studies in Pharmacy, University of Bradford, Bradford, BD7 1DP*)

A gas-liquid chromatographic method for the determination of balsamic acids and esters in crude drugs is described. Methylation of free acids is followed by a single-stage separation and quantitative determination of methyl and benzyl esters of benzoic and cinnamic acids. Results are presented for the column parameters and reproducibility of the method. The analysis of a commercial sample of tolu balsam is reported; the interpretation of the results may offer additional information to that obtained from official standards.

OFFICIAL methods for the evaluation of balsamic drugs rely upon the determination of total balsamic acids, together with acid, ester and saponification values, which are normally calculated with reference to the dry alcohol-soluble matter.[1,2] These standards yield limited information on the drug and do not enable sophisticated products to be readily detected. Additionally, the determination of total balsamic acids presents practical difficulties owing to the resinous nature of these drugs,[3,4] and the analysis of individual acids can be achieved only after bromination of the cinnamic acid fraction.

More recent procedures based on thin-layer chromatography do not permit the satisfactory resolution of acids and esters on a single system and are difficult to make quantitative.[5–7] Gas-liquid chromatography is a convenient method for the evaluation of the free acids and esters, but the separation of free benzoic and cinnamic acids is difficult owing to tailing, as found by Monard and Grenier,[7] although more efficient separations can be achieved by esterification of these acids.[8,9]

This paper describes the development of a gas-liquid chromatographic method for the determination of free balsamic acids as their methyl esters together with the benzyl esters in a commercial sample of tolu balsam.

## EXPERIMENTAL

### REAGENTS AND MATERIALS—

All reagents and standards were of general laboratory grade, and their purities were verified by gas-liquid chromatography on the system described below.

*Diazomethane*—A solution in ethyl acetate was prepared from nitrosomethylurea.[10]

*Internal standard solution*—A 0·6 per cent. *m/V* solution of n-hexadecane in ethyl acetate was used.

### APPARATUS—

*Gas chromatograph*—A Pye, Model 104, gas chromatograph was used, with a flame-ionisation detector maintained at the temperature of the column.

*Glass column*—The column, of dimensions 2·7 m × 3 mm i.d., packed with 10 per cent. E301 on Diatomite C (acid washed and dimethyldichlorosilanised, 90 to 100 mesh, supplied by J. J. Chromatography Ltd.), was conditioned at 300 °C for 24 hours before use. In order to prevent blockage of the column by resin from the balsam, the injection port was lightly plugged with glass-wool, which was replaced daily.

*Integrator*—Peak areas were determined by means of a Honeywell digital integrator.

*Operating conditions*—The initial column temperature was 175 °C for 1 minute, programmed at the rate of 9 °C min⁻¹ to a final temperature of 240 °C for 8 minutes. The initial injection block temperature was 220 °C and the final temperature 260 °C.

The carrier gas was nitrogen at the flow-rate of 80 ml min⁻¹.

The amplifier attenuation was 2 × 10⁻⁸ A (full scale). For the integrator, a threshold value of 3 per cent. was found to be necessary owing to slight base-line drift that occurred at the higher temperature.

SAMPLE PREPARATION—

*Standard solutions*—Methylation of benzoic (25 to 120 mg) and cinnamic acids (25 to 160 mg) was achieved by the careful addition of diazomethane solution at 0 °C. The products were transferred into a 100-ml standard flask, known amounts of benzyl benzoate (40 to 220 mg) and benzyl cinnamate (50 to 450 mg) were added together with 25 ml of internal standard solution, and the volume was made up with ethyl acetate.

*Tolu balsam solution*—Approximately 1 g of tolu balsam was accurately weighed into a 50-ml beaker and diazomethane solution slowly added until effervescence ceased. The mixture was stirred continuously during the methylation process so as to facilitate dissolution of the resinous material and to ensure efficient release of the free acids contained in the sample. After allowing the beaker to stand for 15 minutes, the contents were transferred into a 100-ml standard flask, 25 ml of internal standard solution added, and the mixture was diluted to volume with ethyl acetate.

GAS CHROMATOGRAPHY—

For calibration, 6 μl of standard solution were injected and the temperature programme was started simultaneously; this volume gave approximately 80 per cent. of full-scale deflection for the peak due to the internal standard. Areas under the peaks were measured and standard ratios were calculated with reference to the internal standard. These solutions were used to check the linearity of the system. For the analysis of balsam, one standard solution was adopted and single injections of this solution were interspersed between duplicate 6-μl injections of balsam test solution. Test ratios for acids and esters in the balsam were calculated with reference to the internal standard peak.

Contents of free acids and esters were calculated from the following equation:

$$\text{Content (per cent.)} = \frac{\text{Test ratio}}{\text{Standard ratio}} \times \frac{\text{Mass of standard in 100 ml (g)}}{\text{Mass of sample (g)}} \times 100$$

RESULTS AND DISCUSSION

Typical gas-liquid chromatograms for a standard solution and a balsam solution are shown in Fig. 1. Temperature programming was found to be necessary for adequate resolution of the methyl benzoate peak from the solvent peak and at the same time permitting the determination of the major components in a single analysis. Additionally, the peaks were
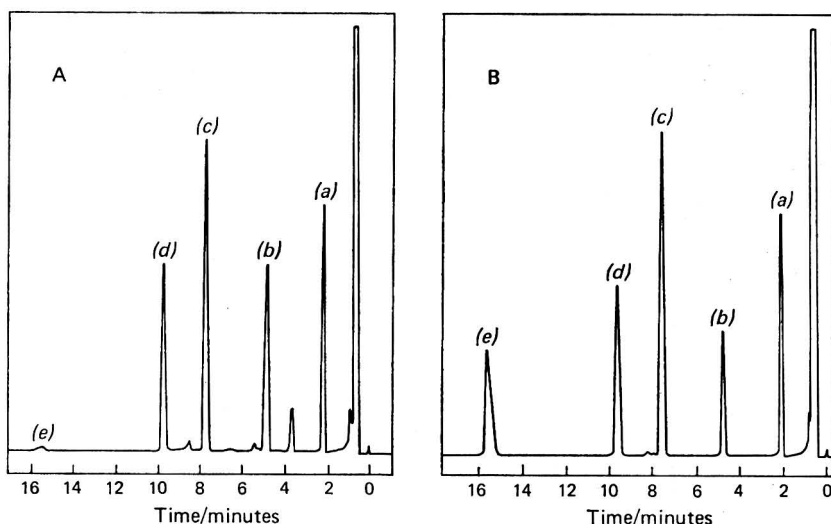


Fig. 1. Chromatogram A, commercial sample of tolu balsam; chromatogram B, standard solution. (*a*), Methyl benzoate; (*b*), methyl cinnamate; (*c*), n-hexadecane; (*d*), benzyl benzoate; and (*e*), benzyl cinnamate

resolved more satisfactorily than was found to be possible with isothermal operation, eliminating interference from minor components in the balsam sample.

Several stationary phases of different polarity groups were investigated; in general, relatively non-polar packings were found to be more suitable and of these the E301 silicone elastomer gave the optimum resolution on a 2·7-m column. Column parameters are given in Table I.

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TABLE I

COLUMN PARAMETERS

</div>

| | | | | Retention time/minutes | Efficiency (No. of theoretical plates) |
|---|---|---|---|---|---|
| Methyl benzoate | .. | .. | .. | 2·2 | 1940 |
| Methyl cinnamate | .. | .. | .. | 4·9 | 7430 |
| n-Hexadecane .. | .. | .. | .. | 7·7 | 12 460 |
| Benzyl benzoate | .. | .. | .. | 9·7 | 16 640 |
| Benzyl cinnamate | .. | .. | .. | 15·5 | 10 680 |
| Benzoic acid .. | .. | .. | .. | 2·4 | — |
| Cinnamic acid .. | .. | .. | .. | 5·4 | — |

The conversion of the free acids into their methyl esters was found to be advantageous, giving high column efficiencies and a marked increase in detector sensitivity, without the tailing that is commonly associated with organic acids.[7,11] Diazomethane was selected in preference to other methylating reagents because of the simple procedure required for its use in esterification, the absence of interfering products and the obviation of the need for solvent extraction of the reaction mixture, so reducing the possible sources of experimental error in the quantitative determination. Difficulties in the use of this reagent lay chiefly in the preparation and storage of the starting material, although these difficulties were minimised by storing batches of the reagent in a deep-freeze for several weeks. The completeness of the reaction was checked by the absence of peaks for benzoic and cinnamic acids (Table I). However, it was found that a large excess of diazomethane in the final solution caused a secondary reaction with methyl cinnamate, producing a compound with a retention time of 10·0 minutes, and hence gave unreliable values for the cinnamic acid content of the balsam. The nature of the secondary reaction is under investigation. The use of boron trifluoride in methanol[12] was investigated; although gas chromatography could be carried out directly on the diluted reaction mixture, the presence of even a slight excess of methanol caused severe tailing of the solvent peak and loss of resolution of methyl benzoate.

The reproducibility of the instrument was verified by repeated injections of a single standard solution; standard ratios (peak area for the solute to peak area for the internal standard) were calculated (Table II). Calibration graphs prepared by using several standard solutions of different concentrations showed that correlation coefficients for each component were between 0·990 and 0·998, indicating that a linear relationship between amount of substance and detector response existed within the ranges indicated above. As standard solutions were prepared from the free acids, the linearity of the response for their methyl esters showed the methylation reaction to be complete. In order to ascertain whether

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TABLE II

PEAK-AREA RATIOS OBTAINED FROM REPEATED INJECTIONS OF A STANDARD
SOLUTION WITH RESPECT TO n-HEXADECANE AS INTERNAL STANDARD

</div>

| Component in solution | | | Amount present/mg | Mean ratio* | Standard deviation | Coefficient of variation, per cent. |
|---|---|---|---|---|---|---|
| Benzoic acid .. | .. | .. | 118·3 | 0·585 | ±0·004 | 0·75 |
| Cinnamic acid .. | .. | .. | 96·5 | 0·966 | ±0·005 | 0·49 |
| Benzyl benzoate | .. | .. | 166·5 | 1·37 | ±0·01 | 0·76 |
| Benzyl cinnamate | .. | .. | 416·6 | 3·25 | ±0·05 | 1·64 |

<div align="center">

* Values obtained from ten injections of the solution.

</div>

methylation of the acids contained in the balsam, in which the matrix is resinous, was also complete, nine replicate amounts of a commercial sample of tolu balsam |were analysed. The results are summarised in Table III.

TABLE III

ANALYSIS OF A COMMERCIAL SAMPLE OF TOLU BALSAM

| Replicate No.* | Benzoic acid, per cent. | | Cinnamic acid, per cent. | | Benzyl benzoate, per cent. | |
|---|---|---|---|---|---|---|
| | Mean | Coefficient of variation | Mean | Coefficient of variation | Mean | Coefficient of variation |
| 1 | 7·7 | 2·6 | 10·5 | 1·0 | 11·4 | 1·6 |
| 2 | 7·4 | 1·6 | 11·3 | 1·6 | 11·5 | 1·9 |
| 3 | 7·9 | 2·9 | 10·9 | 1·0 | 11·4 | 0·8 |
| 4 | 7·8 | 0·8 | 11·3 | 1·2 | 11·3 | 1·2 |
| 5 | 7·8 | 0·8 | 11·2 | 0·7 | 11·5 | 0·9 |
| 6 | 7·8 | 0·7 | 11·2 | 1·2 | 11·7 | 3·1 |
| 7 | 7·8 | 2·1 | 11·1 | 0·6 | 11·4 | 2·4 |
| 8 | 7·7 | 0·8 | 10·3 | 0·3 | 11·3 | 0·5 |
| 9 | 7·7 | 1·3 | 11·4 | 2·0 | 11·4 | 3·1 |
| Over-all mean | 7·8 | 1·3 | 11·0 | 1·1 | 11·4 | 1·7 |

* Results calculated from four determinations on each replicate amount.

The sample of tolu balsam used in this work was found to contain only a trace amount of benzyl cinnamate, so that an accurate quantitative determination of this component could not be made without amending the experimental conditions. This finding agrees with constituents listed in the official monograph for Tolu Balsam B.P.C.[13] However, current investigations indicate that measurable amounts of this ester occur in authentic museum samples of the drug and can be determined by this method.

The results given in Table III show the method to be reliable and reproducible for the analysis of tolu balsam. The chromatographic determination of free balsamic acid and ester components of the drug offers a significant advantage over the official standards, particularly as the latter include the acid and ester components of the resin, which have little or no therapeutic value. A detailed study of several samples of the drug and comparison with official values is the subject of current work and will be reported later.

In addition to benzyl cinnamate, other trace constituents can be determined simultaneously, and may be important in detecting sophistication of the drug. Preliminary studies indicate that the method can readily be applied to the analysis of other balsamic drugs.

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# Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

# Application of Gas-Liquid Chromatography to the Analysis of Essential Oils

## Part III.* The Determination of Geraniol in Oils of Citronella

THE Analytical Methods Committee has received the following report from its Essential Oils Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

### REPORT

The constitution of the Essential Oils Sub-Committee responsible for the preparation of this Report was: Mr. A. M. Humphrey (Chairman), Mr. J. H. Greaves, Mr. B. E. Kent, Mr. W. S. Matthews, Mr. R. G. Perry, Mr. J. Ridlington, Mr. R. A. Stocks and Mr. G. Watson, with Mr. P. W. Shallis as Secretary.

### INTRODUCTION

Geraniol, a primary alcohol ($C_{10}H_{18}O$), occurs widely in essential oils and is the major constituent of oil of palmarosa. It is usually associated with trace amounts of its geometric isomer, nerol, and with other terpenic alcohols. This factor has made its determination in essential oils a difficult procedure. In the case of oil of citronella the geraniol content is of particular importance as this content is used as a basis for the determination of the quality and commercial value of the oil. The alcohols in an essential oil are usually determined by acetylation, but in the case of oil of citronella the citronellal is also affected by acetylation and gives rise to a falsely high result for the true geraniol content. This difficulty has been recognised for many years and to express this apparent "alcohol" content the term "total geraniol" has been adopted. Consistent values for this "total geraniol" are obtained only by the most careful work, as a slight variation in the acetylation procedure affects the degree of acetylation of the citronellal, and although procedures have been suggested for the subtraction of the citronellal contribution there is still a further unknown contribution from other alcohols known to be present, *e.g.*, nerol, linalol, isopulegol and citronellol.

In view of this unsatisfactory situation, the Essential Oils Sub-Committee decided to examine the possibility of using gas-liquid chromatography for the determination of the true geraniol contents of oils of citronella.

### HISTORICAL REVIEW

In 1896, Umney[1] was the first to propose that the geraniol content of oil of citronella could be related to its quality. The method used for its determination was the Liebermann acetylation procedure[2] with acetic anhydride and sodium acetate. The most favourable conditions for this determination were established by Messrs. Schimmel & Co.[3] and are ostensibly the same as those in current use.[4,5] However, in 1909, Semmler[6] showed that citronellal behaves towards acetic anhydride as a substituted vinyl alcohol, and according to him forms citronellal enol acetate, which is subsequently converted into isopulegyl acetate. He also noted the formation of a diacetate, which was confirmed by de Jong in 1919[7] and further confirmed by Reclaire and Spoelstra[8] in 1927. The latter workers further noted the variability of the results obtained by the acetylation procedure and attributed this variability to the non-stoicheiometric reaction of the acetic anhydride with the citronellal. In their paper they suggested that the true geraniol content could be determined after the citronellal had been converted into its oxime by the method of Dupont and Labaune,[9,10] in which aqueous hydroxylamine solution is used.

* For particulars of Part II of this series, see *Analyst*, 1973, **98**, 616.

The situation was reviewed in 1932 by Zimmermann,[11] who showed that the oximation procedure was not quantitative and further that some hydrolysis of the oxime occurred during the saponification of the acetylated oil. His work confirmed the formation of mono- and diacetates of citronellal and he suggested that the true geraniol content could be determined by a phthalisation procedure.[12] It was left to Sabetay and Naves in 1939[13] to publish details of a modified procedure in which phthalic anhydride and pyridine are used. However, the method is seldom used for commercial analyses.

As an alternative to the acetylation method the use has been proposed of the "hot formylation" method of Glichitch,[14] but it was shown by Holness[15] that this procedure also suffered from anomalies similar to those of the acetylation procedure when applied to oil of citronella.

Having reviewed the complexities of the previous work on this subject, the Essential Oils Sub-Committee undertook a preliminary examination of the gas - liquid chromatography of oil of citronella with a view to ascertaining the feasibility of applying the method so as to obtain more specific results for the geraniol content.

EXPERIMENTAL

A sample of oil of Java citronella was circulated to members of the Sub-Committee for gas-chromatographic examinations and seven members submitted results. Each participant was asked to devise his own conditions for the test. All except one used the method of internal standards, and in all instances the instrument was calibrated by using one or more mixtures of internal standard and high-purity geraniol.

A summary of the conditions used by participating laboratories is shown in Table I. It will be seen that a wide variety of instruments and conditions were used. In some instances, the homogeneity of the geraniol peak component was checked by trapping and testing it by infrared spectroscopy or thin-layer chromatography.[16]

TABLE I

GAS-CHROMATOGRAPHIC CONDITIONS USED BY COLLABORATING LABORATORIES

| Laboratory | | | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| Instrument* | | .. | Pye 104 | P.E. F-11 | P.E. 880 | Pye 104 | P.E. F-11 | Pye 104 |
| Column length | | .. | 9 feet | 2 m | 6 feet | 9 feet | 8 feet | 7 feet |
| Support | | .. | Celite, 100 to 120 mesh | Celite, 60 to 80 mesh | Chromo-sorb W, 80 to 100 mesh | Chromo-sorb W, 80 to 100 mesh | Chromo-sorb W, 80 to 100 mesh | Gas-Chrom Q |
| Stationary phase | | .. | Apiezon L | Carbowax 20M | Carbowax 20M | Silicone DC 710 | Butane-1,4-diol succinate | Poly-ethylene glycol adipate |
| Stationary phase loading | | | 10% + 1% FFAP | 5% | 15% | 7·5% | 10% | 10% |
| Column temperature/°C | | | 120 | 130 | 140 | 175 | 115 | 135 |
| Injection temperature/°C | | | On-column | 220 | 200 | On-column | 140 | On-column |
| Sample size/$\mu$l | | .. | 0·1 | 0·2 | 2·0 (dilute) | 0·16 | 0·1 | 0·2 |
| Chart speed/in h$^{-1}$ | | .. | 6 | 12 | 12 | 60 | 12 | 15 |
| Internal standard | | .. | Diphenyl-methane | 2-Phenyl-ethanol | None† | Butyl benzoate | 2-Phenyl-ethanol | Eugenol |

* With flame-ionisation detector in each instance.
† External standardisation used.

RESULTS

The results obtained in six participating laboratories by use of various methods of peak measurements are summarised in Table II. These results show that the sample of oil of citronella probably contained between 22 and 25 per cent. of geraniol, a much lower figure than may be implied from the usual results of chemical analysis by an acetylation procedure. The variation between laboratories provides a useful guide to the differences that may be expected between laboratories that use gas - liquid chromatography, but without any standardisation of conditions.

TABLE II

DETERMINATION OF GERANIOL IN OIL OF JAVA CITRONELLA

Geraniol found, per cent. $m/m$, by*—

| Laboratory | (a) | (b) | (c) | (d) |
|---|---|---|---|---|
| A† | — | 21·8 | 25·4 | 24·5 |
| B | 22·4, 22·4, 22·6 | 22·6, 22·6, 22·6 | 23·0, 23·0, 22·9 | — |
| C‡ | — | 24·8, 24·8 | 24·8, 24·8 | 24·8, 24·8 |
| D | — | 22·8, 22·2, 23·6 | 21·7, 23·8, 22·8 | 21·7, 23·6, 22·6 |
| E | 25·1, 24·5, 24·0 | — | — | — |
| F | 23·0 | 23·3 | 23·6 | — |

* Method of calculating peak "area"—

    (a)  Integration;
    (b)  Peak height × width at half-height;
    (c)  Peak height × retention distance;
    (d)  Peak height only.

† Each result is the mean of five determinations.
‡ Results obtained by a method of external standardisation.

It was concluded that gas - liquid chromatography could serve as a basis for a reliable determination of geraniol in oil of citronella and it was also evident that a variety of instruments, columns and conditions could be used for this determination.

Consideration of the results obtained thus far led to the adoption of a standardised procedure in which Carbowax 20M is used as a stationary phase and 2-phenylethanol as the internal standard. The homogeneity of the geraniol peak component so obtained was again checked by infrared spectroscopy, mass spectrometry and coupled thin-layer and gas - liquid chromatography. Several samples of different oils of citronella were circulated to the collaborating members, who were asked to determine the geraniol contents according to the following method.

*Preparation of calibration mixture*—Accurately weigh about 1 g of geraniol and 1 g of 2-phenylethanol and dilute to 100 ml with ethyl acetate. The geraniol used should be at least 99 per cent. pure.

*Preparation of sample*—Accurately weigh 4 g of the oil under examination and 1 g of 2-phenylethanol and dilute to 100 ml with ethyl acetate.

*Gas-chromatographic conditions*—Column length, 6 feet; column packing, 15 per cent. Carbowax 20M on Chromosorb W, 85 to 100 mesh, acid washed and dimethyldichlorosilanised; flow-rate for $\frac{1}{8}$-inch column, 40 ml min⁻¹; carrier gas, nitrogen; isothermal temperature, 140 °C; and sample size, 1 to 5 $\mu$l, to suit attenuation setting.

From the peak height × retention distance, determine a mean response factor, $f$, for geraniol to 2-phenylethanol by using the equation—

$$f = \frac{\text{2-phenylethanol response} \times \text{mass of geraniol}}{\text{geraniol response} \times \text{mass of 2-phenylethanol}}$$

and use this factor to calculate the percentage of geraniol in the samples by using the equation—

$$\text{Geraniol, per cent.} = f \times \frac{\text{geraniol response} \times \text{mass of 2-phenylethanol} \times 100}{\text{2-phenylethanol response} \times \text{mass of sample}}$$

NOTE—

The results in Table II support the evidence provided in Part I[17] of this series on the suitability of this method of measurement of relative peak response.

Six different oils were circulated: two samples of Ceylon oil, two of Formosan and one each of Java and Chinese. A summary of the results obtained is given in Table III.

Each of the collaborating members in this exercise reported a good precision, but criticised the use of 2-phenylethanol as an internal standard when applied to the sample of Ceylon oil B, owing to the presence of an interfering peak. An alternative internal standard was sought, and n-dodecanol was found to be suitable. The exercise was repeated, replacing the 2-phenylethanol by n-dodecanol and restricting the determinations to two oils only,

*viz.*, Ceylon B and Java B oils. In Table IV is a summary of the results obtained, which includes details of the columns used. Again, each of the collaborating members reported a good precision and there were no difficulties in the use of the n-dodecanol as the internal standard.

### TABLE III
#### DETERMINATION OF GERANIOL (PER CENT.) IN VARIOUS OILS OF CITRONELLA
Internal standard: 2-phenylethanol

| Laboratory | Ceylon A | Ceylon B | Formosan A | Formosan B | Java B | Chinese |
|---|---|---|---|---|---|---|
| A | 16·8 | 16·9 | 17·6 | 21·5 | 19·9 | 21·8 |
| C | 17·9 | 18·5 | 17·9 | 21·0 | 20·2 | 22·1 |
| D | 18·0 | 18·6 | 18·0 | 20·9 | 21·3 | 22·4 |
| E | 17·3 | 17·0 | 17·4 | 22·2 | 21·6 | 22·9 |
| F | 17·9 | 18·2 | 18·1 | 21·1 | 20·9 | 23·1 |
| G | 17·5 | 17·9 | 18·0 | 21·3 | 21·2 | 23·1 |

Typical traces for the four different types of oils are shown in Figs. 1 to 4.

These two sets of results can be compared with the corresponding results in Table III but it is to be noted that they should not be compared with the results in Table II as these applied to a different sample of oil of Java citronella.

IDENTIFICATION OF THE GERANIOL PEAK—

An initial identification of the peak was made by the use of retention data followed by the comparison of an oil with a sample of the same oil spiked with geraniol. There was no apparent increase in the peak width compared with its height, thus confirming the retention data. The actual identity of the peak was confirmed by mass spectrometry with a Perkin-Elmer RMU-5 mass spectrometer, which also confirmed its homogeneity. This observation was further confirmed by applying a coupled thin-layer and gas-chromatographic technique.[16]

A sample of fortified oil was assayed for geraniol and comparison of the results with the original oil showed a recovery of 94 per cent.

VARIATIONS IN LABORATORY WORKING CONDITIONS—

In the final stages of the exercise it can be seen that some variation in the operating conditions still existed (see Table IV). These variations are due primarily to the different constructional details of the gas chromatographs used and may be grouped under four headings.

### TABLE IV
#### DETERMINATION OF GERANIOL (PER CENT.) IN TWO OILS OF CITRONELLA
Internal standard: n-dodecanol

| Laboratory | Ceylon B | Java B | Stationary phase loading, per cent. | Flow-rate/ ml min$^{-1}$ | Column diameter/ inch | Injection |
|---|---|---|---|---|---|---|
| A | 16·2 | 20·0 | 15 | 60 | ¼ | On-column |
| B | 17·3 | 19·8 | 15 | 22 p.s.i. | ⅛ | Flash, 220 °C |
| C | 18·4 | 21·5 | 15 | 40 | ⅛ | Flash, 200 °C |
| D | 17·8 | 20·4 | 5 | 40 | ¼ | On-column |
| E | 17·8 | 20·6 | 15 | 20 p.s.i. | ⅛ | Flash, 150 °C |
| F | 18·3 | 20·7 | 10 | 50 | ¼ | On-column |
| G | 17·8 | 21·4 | 10 | 40 | ¼ | Flash, 200 °C |
| H | 18·3 | 20·9 | 15 | 40 | ⅛ | Flash, 150 °C |

*Stationary phase loading*—Although specified in this collaborative work at 15 per cent., the availability of columns with a different loading may make them an attractive alternative. In the case of laboratory D, the lower loading was used in order to reduce the retention time. This laboratory was using a ¼-inch glass column and the flow-rate of 40 ml min$^{-1}$ gave unduly high retention times with a 15 per cent. loading. Laboratory H also reported long retention times, but managed to achieve results with a 10 per cent. loading. Laboratory A was able to use a 15 per cent. loading with a ¼-inch glass column because of the higher flow-rate.
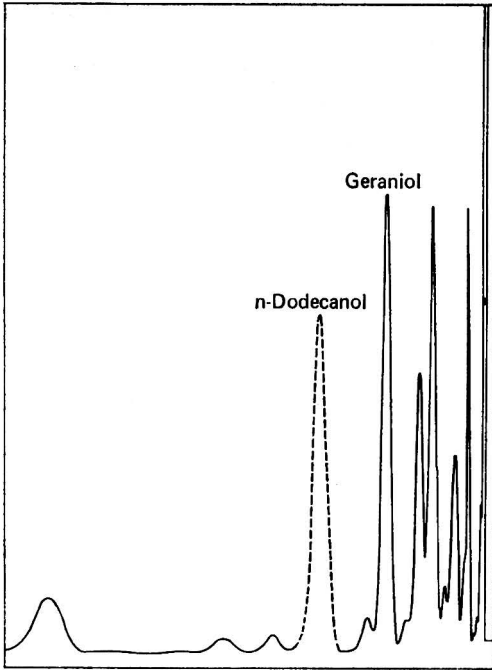
Fig. 1.  Typical  chromatogram  of  Ceylon citronella oil, with n-dodecanol as internal standard. Total analysis time 30 minutes
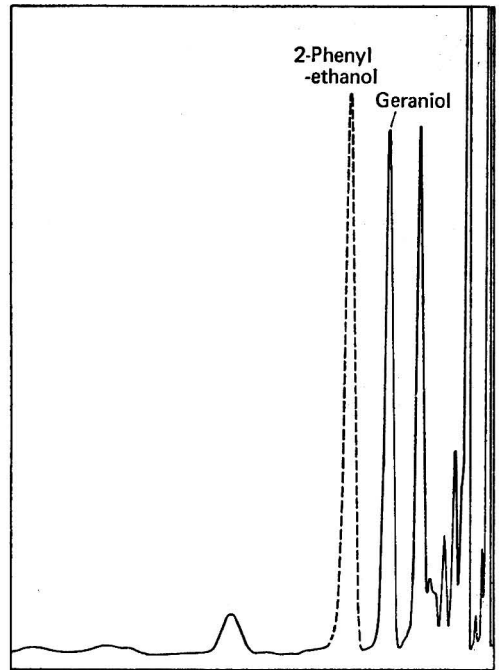


Fig. 2.  Typical chromatogram of Java citronella oil, with 2-phenylethanol as internal standard. Total analysis time 30 minutes
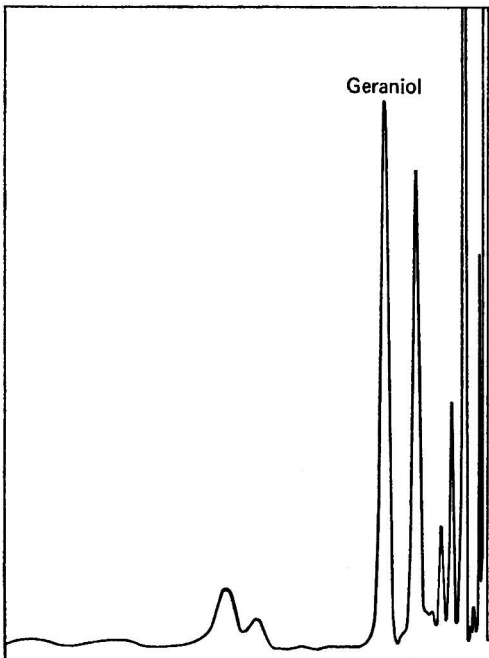


Fig. 3.  Typical  chromatogram  of  Chinese citronella oil.   Total analysis time 30 minutes
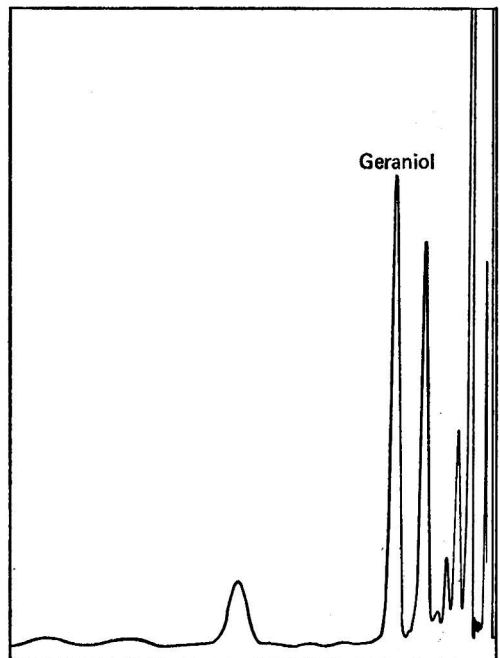


Fig. 4.  Typical  chromatogram  of  Formosan citronella oil.   Total analysis time 30 minutes

*Carrier flow-rate*—This can be optimised for any particular column in order to give the maximum resolving power, but it is often altered so as to give the desired retention time, particularly if it is undesirable to alter the temperature.   Measurement of the flow-rate is not always easy or convenient and some laboratories give the inlet pressure.

*Column diameter*—This is usually fixed by the design of the instrument and it is often found that glass columns are ¼ inch in external diameter whereas stainless-steel columns are ⅛ inch in external diameter.   Other variations are also known to exist.   The wider-bore columns have a lower pressure drop and require higher flow-rates than an equivalent column of smaller diameter in order to achieve similar retention times.

*Injection system*—This can either be "on column" or "flash" and the results did not show that either method was inferior to the other.

In general, it was found that the variations shown in Table IV did not affect the results, but it must be borne in mind that the stationary phase loading, the flow-rate and the column dimensions are all inter-related so far as the retention time is concerned, and widely different variations of these factors may adversely affect the resolution.

There was no evidence that an injector temperature of 200 °C had any adverse effects and no degradation was apparent from the use of stainless-steel columns.   The use of acid-washed, dimethyldichlorosilanised Chromosorb W was found to be satisfactory and no attempts were made to use more sophisticated supports.

## Conclusions

The quantitative determination of geraniol in commercially available samples of oils of citronella can be satisfactorily achieved by a gas - liquid chromatographic procedure. Based on experience with a range of oils and operating conditions, the Sub-Committee recommends the procedure given in the Appendix, which should provide accurate results for the determination of the geraniol content with an acceptable level of precision, and should reduce variations, both within and between laboratories, to a satisfactory level.

## Appendix

### Recommended method for the determination of geraniol in oils of citronella by gas - liquid chromatography

OPERATING CONDITIONS—

It is essential that throughout a determination the operating conditions are maintained as constant as practicable.   It is also essential to use the detector - amplifier system within its linear range[18]:

| | |
|---|---|
| Detector .. .. .. | Flame ionisation |
| Stationary phase .. | Carbowax 20M |
| Support .. .. .. | Chromosorb W, acid washed, dimethyldichlorosilanised, 80 to 100 mesh |
| Stationary phase loading | About 10 per cent. *m/m* |
| Column .. .. .. | 5 to 9 feet.   Outside diameter ⅛ to ¼ inch, glass or stainless steel |
| Column temperature .. | Isothermal, 140 °C |
| Injection .. .. | On-column or flash between 150 and 200 °C |
| Chart speed .. .. | 6 in h$^{-1}$ (minimum) |
| Internal standard .. | n-Dodecanol.   Purity by gas - liquid chromatography not less than 99 per cent. |
| Geraniol .. .. .. | Purity by gas - liquid chromatography not less than 99 per cent. |
| Sample size .. .. | Such that the internal standard and geraniol peaks fall within the linear range |
| Peak heights .. .. | Internal standard and geraniol, within 40 to 75 per cent. of full-scale deflection |
| Gas flow-rates .. .. | To give satisfactory instrument performance |
| Solvent .. .. .. | Ethyl acetate |

DETERMINATION OF THE FACTOR, *f*, FOR THE INTERNAL STANDARD (GERANIOL = 1)—

Make all weighings to an accuracy of 0·2 mg.

Weigh about 1·0 g of geraniol and 1·0 g of n-dodecanol (or such other amounts as will give approximately equal heights for the two peaks), and dissolve them in about 100 ml of solvent.   Inject 1·0 $\mu l$, or such other volume of the solution as will ensure response within the linear range, and calculate to three decimal places the factor, *f*, from the equation

$$f = \frac{h \times d}{h_g \times d_g} \times \frac{W_g}{W}$$

where $h_g$ is the height of the geraniol peak, $d_g$ the retention distance of the geraniol peak, $h$ the height of the n-dodecanol peak, $d$ the retention distance of the n-dodecanol peak, $W$ the amount of n-dodecanol and $W_g$ the amount of geraniol.

Repeat the operation twice on the same solution, and use the average of the three values of $f$ in the calculation of the geraniol content of the sample.

DETERMINATION OF THE GERANIOL CONTENT OF THE SAMPLE—

Weigh about 4 g of sample and an amount of n-dodecanol (usually about 1 g) that will give approximately equal heights for the two peaks of interest and dissolve them in about 100 ml of solvent. Inject $1 \cdot 0 \,\mu$l, or such other volume as will ensure response within the linear range, and calculate the geraniol content of the sample to two decimal places from the equation

$$\text{Geraniol, per cent.} = f \times \frac{H_g \times D_g}{H \times D} \times \frac{W}{W_s} \times 100$$

where $H_g$ is the height of the geraniol peak, $D_g$ the retention distance of the geraniol peak, $H$ the height of the n-dodecanol peak, $D$ the retention distance of the n-dodecanol peak, $W$ the amount of n-dodecanol, $W_s$ the amount of sample and $f$ the average response factor.

Repeat the operation twice with the same solution, and report the average of the three results to one decimal place.

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    NOTE—Reference 17 is to Part I of this series.

# Recommended Methods for the Evaluation of Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL
SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON
RECOMMENDED METHODS FOR THE EVALUATION OF DRUGS

## The Chemical Assay of Cascara Bark and Cascara Dry Extract

IN 1968, Panel 3 of the Joint Committee of the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry published recommended methods for the chemical assay of cascara dry extract, cascara tablets and cascara bark.[1] Subsequently, the European Pharmacopoeia[2] has included a modified version of the method recommended by the Joint Committee for the assay of cascara bark. In consequence, the Joint Committee set up a Panel to compare the two different methods and to report its findings. The work carried out by this Panel and the conclusions it has drawn are given in this report. The Constitution of the Panel was: Dr. D. C. Garratt (Chairman), Dr. C. Daglish, Mr. J. D. Edmond, Professor J. W. Fairbairn, Mr. S. C. Jolly, Dr. P. A. Linley, Mr. N. Nix, Mr. C. A. Macdonald, Mr. M. H. Ransom, Mrs. S. Richens and Mr. F. H. Tresadern, with Mr. P. W. Shallis as Secretary.

### Cascara Bark

#### EXPERIMENTAL

The method recommended by the Joint Committee is based on that described by Fairbairn and Simic.[3] In this method preliminary extraction of the dry powdered bark is carried out with 70 per cent. ethanol, from which the free anthraquinones are extracted with carbon tetrachloride and the aloins and cascarosides are partitioned between ethyl acetate and water. The aloins are then recovered from the ethyl acetate phase, oxidised with iron(III) chloride, the product is extracted with carbon tetrachloride, transferred to sodium hydroxide solution, and the resulting aglycones are determined by measuring the extinction at the maximum at about 500 nm. The cascarosides are determined in a similar way in the aqueous phase, and the concentrations of aloins and cascarosides present in the sample are calculated, as cascaroside A, from an established $E_{1\,cm}^{1\%}$ value at 500 nm of the red solution obtained from pure cascaroside A under the same conditions.

The method included in the European Pharmacopoeia[2] differs from the Joint Committee's method in two main respects. First, the preliminary extraction is carried out with hot water instead of with 70 per cent. ethanol, and secondly, the transfer of the final product to sodium hydroxide solution is made by evaporating the carbon tetrachloride solution to dryness and dissolving the residue in 1 N sodium hydroxide, whereas in the Joint Committee's method the product is extracted from the carbon tetrachloride with 1 N sodium hydroxide.

The Panel decided to carry out a collaborative comparison of the two methods, and this comparison was extended to include the other two possible combinations of the methods of preliminary extraction with the alternative methods of final transfer of the product to sodium hydroxide solution. The requirement of the European Pharmacopoeia method that the extinction of the final coloured solution should be measured within 1 hour of making the sodium hydroxide solution up to volume was thought by members of the Panel to be a possible source of major variations, as in the original work of Panel 3 the final coloured solution had been found to be unstable in daylight. In consequence, it was decided that in the collaborative work extinctions should, for the purposes of comparison, be made within 5 minutes of adjusting the final solution to volume and again after 1 hour.

In order to indicate possible contamination of the solutions containing aloins and cascarosides, both methods under investigation incorporate an additional extinction measurement at 440 nm; the ratio of the extinction at 440 nm to that at 500 nm is then taken as an indication of the validity of the assay result. The lower limiting values for this ratio given in the Joint Committee's method are 1·7 for the determination of aloins and 1·8 for the determination of cascarosides. The European Pharmacopoeia had originally decided that 1·8 should be the limiting ratio value for both determinations. The Panel therefore decided to record all ratio values in its collaborative comparison of the methods in order to ascertain whether this higher value for the determination of aloins was justified.

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The assay included in the European Pharmacopoeia makes use of standardisation against 1,8-dihydroxyanthraquinone, whereas in the Joint Committee's method the assay results are calculated from an established $E_{1\,cm}^{1\%}$ value for pure cascaroside A. Members of the Panel were of the opinion that, as no established reference sample of 1,8-dihydroxyanthraquinone was available, differences between samples of this material could give rise to errors greater than those normally associated with spectrophotometric measurement. It was therefore decided that, for the purposes of the collaborative test, assay results would be calculated by using the known $E_{1\,cm}^{1\%}$ value of 125 for cascaroside A under the same conditions.

A common sample of powdered cascara bark was distributed to all collaborators and assays were carried out in duplicate by both methods and the other two possible combinations of the conditions. The assay results and the individual values for the ratios of the extinctions at 440 nm and at 500 nm are given in Tables I and II, respectively.

TABLE I

COMPARISON OF RESULTS FOR THE ASSAY OF CASCARA BARK
BY FOUR DIFFERENT METHODS

Methods—

1: Joint Committee's method.[1]

2: European Pharmacopoeia method.[2]

3: Joint Committee's method, but with evaporation of final carbon tetrachloride solution before transfer to sodium hydroxide solution.

4: European Pharmacopoeia method, but with extraction of final carbon tetrachloride solution with 1 N sodium hydroxide.

| Method | Laboratory | Aloins, as cascaroside A, per cent. | | Cascarosides, as cascaroside A, per cent. | | Total glycosides, as cascaroside A, per cent. | |
|---|---|---|---|---|---|---|---|
| | | 5 minutes | 1 hour | 5 minutes | 1 hour | 5 minutes | 1 hour |
| 1 | A | 3·40 | 2·99 | 6·12 | 5·90 | 9·52 | 8·89 |
| | | 3·54 | 3·15 | 6·13 | 5·94 | 9·67 | 9·09 |
| | B | 3·56 | 3·53 | 5·62 | 5·5 | 9·18 | 9·03 |
| | | 3·55 | 3·33 | 6·07 | 6·06 | 9·62 | 9·6 |
| | C | 3·82 | 3·69 | 6·47 | 6·29 | 10·29 | 9·98 |
| | | 3·90 | 3·85 | 6·34 | 6·24 | 10·24 | 10·09 |
| | D | 3·72 | 3·57 | 6·47 | 6·07 | 10·19 | 9·64 |
| | | 3·55 | 3·48 | 6·55 | 6·28 | 10·10 | 9·76 |
| | E | 2·72 | 2·68 | 4·53 | 4·43 | 7·25 | 7·11 |
| | | 3·22 | 3·08 | 5·64 | 5·52 | 8·86 | 8·60 |
| | F | 3·17 | 3·00 | 5·63 | 5·31 | 8·80 | 8·31 |
| | | 3·05 | 2·90 | 5·61 | 5·41 | 8·66 | 8·31 |
| | G | 3·85 | 3·77 | 6·16 | 6·08 | 10·01 | 9·85 |
| | | 3·67 | 3·52 | 6·11 | 6·09 | 9·78 | 9·61 |
| | Mean .. .. .. | 3·48 | 3·32 | 5·96 | 5·79 | 9·44 | 9·13 |
| | Standard deviation .. | 0·337 | 0·359 | 0·529 | 0·514 | 0·837 | 0·834 |
| | Coefficient of variation, per cent. .. .. | 9·68 | 10·80 | 8·87 | 8·87 | 8·87 | 9·13 |
| | 1-Hour means* .. | 100 | 95·4 | 100 | 97·1 | 100 | 96·7 |
| 2 | A | 3·51 | 3·43 | 6·38 | 5·33 | 9·89 | 8·76 |
| | | 3·43 | 3·34 | 6·39 | 5·29 | 9·82 | 8·63 |
| | B | 2·54 | 2·48 | 6·5 | 6·21 | 9·04 | 8·89 |
| | | 2·61 | 2·57 | 5·79 | 5·89 | 8·4 | 8·46 |
| | C | 3·67 | 3·54 | 7·04 | 6·84 | 10·71 | 10·38 |
| | | 3·91 | 3·50 | 7·04 | 6·70 | 10·95 | 10·20 |
| | D | 3·29 | 3·21 | 7·18 | 6·72 | 10·47 | 9·93 |
| | | 3·15 | 3·08 | 7·43 | 7·08 | 10·58 | 10·16 |
| | E | 3·10 | 3·08 | 6·80 | 6·76 | 9·90 | 9·84 |
| | | 3·04 | 3·03 | 6·22 | 5·52 | 9·26 | 8·55 |
| | F | 3·32 | 3·07 | 6·18 | 5·47 | 9·50 | 8·54 |
| | | 3·21 | 2·94 | 6·26 | 5·48 | 9·47 | 8·42 |
| | G | 3·32 | 3·22 | 6·85 | 6·54 | 10·17 | 9·76 |
| | Mean .. .. .. | 3·24 | 3·11 | 6·62 | 6·14 | 9·86 | 9·34 |
| | Standard deviation .. | 0·378 | 0·321 | 0·475 | 0·663 | 0·727 | 0·760 |
| | Coefficient of variation, per cent. .. .. | 11·67 | 10·33 | 7·17 | 10·80 | 7·38 | 8·13 |
| | 1-Hour means* .. | 100 | 96·0 | 100 | 92·7 | 100 | 94·7 |

## TABLE I (*continued*)

| Method | Laboratory | Aloins, as cascaroside A, per cent. | | Cascarosides, as cascaroside A, per cent. | | Total glycosides, as cascaroside A, per cent. | |
|--------|-----------|-----------|--------|-----------|--------|-----------|--------|
| | | 5 minutes | 1 hour | 5 minutes | 1 hour | 5 minutes | 1 hour |
| 3 | A | 3·54 | 2·99 | 6·10 | 6·07 | 9·64 | 9·06 |
| | | 3·60 | 3·06 | 5·93 | 5·83 | 9·53 | 8·89 |
| | B | 3·45 | 3·3 | 5·38 | 5·30 | 8·83 | 8·6 |
| | | 3·33 | 3·15 | 6·32 | 6·27 | 9·65 | 9·42 |
| | C | 3·84 | 3·79 | 7·18 | 7·11 | 11·02 | 10·90 |
| | | 3·74 | 3·65 | 6·65 | 6·57 | 10·39 | 10·22 |
| | D | 3·54 | 3·40 | 6·47 | 6·09 | 10·01 | 9·49 |
| | | 3·48 | 3·40 | 6·70 | 6·47 | 10·18 | 9·87 |
| | E | 3·49 | 3·43 | 6·34 | 6·20 | 9·83 | 9·63 |
| | | 3·28 | 2·92 | 5·81 | 5·36 | 9·09 | 8·28 |
| | F | 3·28 | 3·05 | 5·73 | 5·41 | 9·01 | 8·46 |
| | | 3·17 | 2·98 | 5·63 | 5·31 | 8·80 | 8·29 |
| | G | 3·91 | 3·81 | 6·10 | 5·90 | 10·01 | 9·71 |
| | | 3·72 | 3·58 | 6·10 | 5·87 | 9·82 | 9·45 |
| | Mean .. .. .. | 3·53 | 3·32 | 6·17 | 5·98 | 9·70 | 9·30 |
| | Standard deviation .. | 0·220 | 0·306 | 0·479 | 0·532 | 0·628 | 0·762 |
| | Coefficient of variation, per cent. .. .. | 6·25 | 9·20 | 7·76 | 8·89 | 6·47 | 8·20 |
| | 1-Hour means* .. | 100 | 94·1 | 100 | 96·9 | 100 | 95·9 |
| 4 | A | 3·49 | 3·40 | 6·50 | 5·73 | 9·99 | 9·13 |
| | | 3·45 | 3·36 | 6·45 | 5·79 | 9·90 | 9·15 |
| | B | 2·57 | 2·5 | 6·09 | 5·9 | 8·66 | 8·4 |
| | | 2·59 | 2·59 | 6·08 | 5·72 | 8·67 | 8·31 |
| | C | 3·12 | 3·04 | 6·86 | 6·69 | 9·98 | 9·73 |
| | | 2·98 | 2·90 | 7·19 | 6·63 | 10·17 | 9·53 |
| | D | 3·43 | 3·34 | 7·11 | 6·69 | 10·54 | 10·03 |
| | | 3·36 | 3·28 | 7·25 | 6·98 | 10·61 | 10·26 |
| | E | 2·72 | 2·72 | 7·04 | 7·06 | 9·76 | 9·78 |
| | | 3·72 | 3·69 | 5·68 | 5·21 | 9·40 | 8·90 |
| | F | 3·41 | 3·18 | 5·66 | 5·10 | 9·07 | 8·28 |
| | | 3·41 | 3·24 | 5·75 | 5·18 | 9·16 | 8·42 |
| | G | 3·30 | 3·23 | 6·91 | 6·75 | 10·21 | 9·98 |
| | Mean .. .. .. | 3·20 | 3·11 | 6·50 | 6·11 | 9·70 | 9·22 |
| | Standard deviation .. | 0·370 | 0·347 | 0·598 | 0·716 | 0·655 | 0·714 |
| | Coefficient of variation, per cent. .. .. | 11·59 | 11·14 | 9·19 | 11·72 | 6·75 | 7·75 |
| | 1-Hour means* .. | 100 | 97·2 | 100 | 94·0 | 100 | 95·1 |

\* These figures are the mean values for the results after 1 hour relative to the values obtained for the reading after 5 minutes.

### RESULTS AND DISCUSSION

It can be seen from Table I that the results by both methods and the other two combinations of conditions are very similar, and it is apparent that no advantage is to be gained from the alternative combinations of preliminary extraction and final transfer to sodium hydroxide solution. There is no significant difference between the results by both methods, although it can be seen that the European Pharmacopoeia method gives slightly higher figures for cascarosides and total glycosides. The European Pharmacopoeia method was, however, found to offer certain advantages over the Joint Committee's method in speed and ease of manipulation at some stages. Within-laboratory agreement between results of the duplicate assays is for the most part good and inter-laboratory variations are considered to be no greater than would be expected for a method of this type. The results for total glycosides based on extinction readings taken 1 hour after the final solution had been made up to volume were on average 6 per cent. lower than those based on readings made within 5 minutes, thus indicating that some degradation of the coloured solution had occurred, but no significant differences resulted.

The values for the ratios of extinctions at 500 and 440 nm are given in Table II, and indicate that a limiting value of 1·8 for the determination of cascarosides is satisfactory. However, the Panel is of the opinion that the values for the extinction ratios obtained in

the collaborative work point conclusively to the retention of a limiting value of 1·7 for the determination of aloins, rather than the figure of 1·8 originally proposed by the European Pharmacopoeia.

TABLE II

RATIO OF EXTINCTIONS AT 500 nm TO THOSE AT 440 nm FOR THE READINGS TAKEN WITHIN 5 MINUTES

The key to the methods is as given in Table I

| Method | | Aloins | | | Cascarosides | | |
|---|---|---|---|---|---|---|---|
| | | 500 nm | 440 nm | Ratio | 500 nm | 440 nm | Ratio |
| 1 | Mean* .. .. .. .. | 0·351 | 0·193 | 1·83 | 0·601 | 0·297 | 2·03 |
| | Standard deviation .. .. | | | 0·09 | | | 0·08 |
| | Coefficient of variation, per cent. | | | 5·04 | | | 4·12 |
| 2 | Mean† .. .. .. .. | 0·326 | 0·175 | 1·86 | 0·655 | 0·315 | 2·09 |
| | Standard deviation .. .. | | | 0·10 | | | 0·12 |
| | Coefficient of variation, per cent. | | | 5·47 | | | 5·61 |
| 3 | Mean* .. .. .. .. | 0·356 | 0·192 | 1·86 | 0·623 | 0·301 | 2·08 |
| | Standard deviation .. .. | | | 0·09 | | | 0·10 |
| | Coefficient of variation, per cent. | | | 4·60 | | | 4·71 |
| 4 | Mean† .. .. .. .. | 0·321 | 0·177 | 1·82 | 0·655 | 0·319 | 2·06 |
| | Standard deviation .. .. | | | 0·08 | | | 0·09 |
| | Coefficient of variation, per cent. | | | 4·22 | | | 4·28 |

\* Values are for 14 determinations by 7 laboratories.
† Values are for 13 determinations by 7 laboratories.

One member of the Panel noticed that originally in the European Pharmacopoeia method no instruction was given regarding the method of heating to be used when evaporating the final carbon tetrachloride solution to dryness before dissolving the residue in sodium hydroxide solution. In the Joint Committee's method excessive heating of the final solution is avoided by carrying out the evaporation on a bath of boiling water. To test the effect of the method of heating used for the evaporation of the carbon tetrachloride solution to dryness, the member subjected portions of the carbon tetrachloride solution of an aloins fraction and a cascarosides fraction to heating on a bath of boiling water and on an electric hot-plate. The residue from each was then dissolved in 1 N sodium hydroxide and the extinction measured at 500 nm; the results were as follows:

| | Water-bath | Hot-plate |
|---|---|---|
| Aloins fraction .. .. .. | 0·386 | 0·300 |
| Cascarosides fraction .. .. .. | 0·721 | 0·513 |

From these results the Panel concluded that, as the method of heating used for the evaporation of the carbon tetrachloride could have a profound effect on the extinction of the final alkaline solution, heating on a bath of boiling water should be specified.

The Panel's observations on the European Pharmacopoeia method were passed to the Commission's Group of Experts No. 13 (Pharmacognosy) for consideration. This Group accepted the retention of the ratio value of 1·7 for the determination of aloins and also the requirement that heating of the flask on a bath of boiling water should be specified as the method for evaporation of the carbon tetrachloride solution to dryness. It did not, however, accept that the assay should be standardised against an established $E_{1\text{ cm}}^{1\%}$ value for pure cascaroside A. The Panel is opposed to the introduction of the additional step necessitated by the standardisation of the assay against 1,8-dihydroxyanthraquinone, and is of the opinion that it could be justified only if a reference sample of the standard were made available.

### Cascara Dry Extract

The Panel, having accepted the modifications of the originally recommended method for the assay of cascara bark[1] detailed above, decided that it would be advisable to investigate the possibility of incorporating these modifications in the recommended method for the assay of cascara dry extract. In the Joint Committee's published recommendations the method

for the assay of cascara dry extract differed from that for the assay of cascara bark only in the mass of sample taken and in the volume of water-saturated ethyl acetate used for extraction at the partition stage.

## EXPERIMENTAL

A sample of cascara dry extract was circulated to all the participating laboratories for assay by the procedure as modified to incorporate initial extraction with hot water and transfer of the final product to sodium hydroxide solution after evaporation of the carbon tetrachloride solution to dryness. All laboratories found during the extraction of the free aglycones with carbon tetrachloride that an emulsion formed at the interfacial layer. This emulsion was either impossible or, at best, extremely difficult to break. Attempts to break down the emulsion by the addition of sodium chloride were entirely unsuccessful, continuous centrifugation met with partial success in some laboratories and in one laboratory heating it gently after the addition of a small volume of water afforded some success, but in no instance was all the emulsion broken down by these means. Another laboratory found it possible to avoid the formation of emulsion after initial extraction with hot water by shaking 10 ml of the water extract with 2 drops of 1 N hydrochloric acid, 5 ml of 95 per cent. ethanol and 40 ml of carbon tetrachloride, allowing the layers to separate, extracting the aqueous layer with a further 40 ml of carbon tetrachloride and then proceeding with the ethyl acetate extraction of the aqueous phase in the usual manner. The Panel, however, considered that there was little to choose between this approach and the Joint Committee's original recommendation of an initial overnight extraction with 70 per cent. ethanol.

A further collaborative test involving two samples of cascara dry extract was then carried out. On this occasion the initial extraction was carried out with 70 per cent. ethanol, after which the modified procedure was applied except that the carbon tetrachloride extraction, before the partitioning with ethyl acetate, was carried out with two 40-ml portions of carbon tetrachloride instead of two 20-ml portions, and the volume of ethyl acetate used in the partitioning stage was reduced from 300 to 120 ml. In this work, two laboratories encountered some trouble from the formation of emulsions, which was surprising as this effect had not been evident in the original work carried out for the Joint Committee. The emulsions formed on this occasion were not, however, troublesome, and could be broken down without difficulty. The method as used in this work was considered to be satisfactory and was applied collaboratively to a sample of spray-dried dry extract so as to ensure that the method was satisfactory for the material prepared by different processes. The results obtained on three samples of cascara dry extract are shown in Table III.

## DISCUSSION

The modified procedure for the assay of cascara bark involving initial extraction with hot water gave rise to the formation of intractable emulsions in a subsequent stage of the assay when applied to samples of cascara dry extract. The Panel found that this problem could be overcome by reverting to the use of 70 per cent. ethanol for the initial extraction, as originally recommended. The modified procedure for the transfer of the final product to sodium hydroxide solution was, however, found to be satisfactory for the assay of cascara dry extract and offered some advantage in that it was easier and quicker to perform.

## RECOMMENDATION

The Panel recommends that the methods for the assay of cascara bark and cascara dry extract previously published by the Joint Committee[1] should be replaced by the methods given in Appendixes I and II to this Report.

## Appendix I

### RECOMMENDED METHOD FOR THE ASSAY OF CASCARA BARK

REAGENTS—

*Carbon tetrachloride.*

*Iron(III) chloride, anhydrous, or iron(III) chloride solution*, 60 *per cent.* m/V.

*Hydrochloric acid*, 36 *per cent.* m/m *and* 1 N.
*Methanol.*
*Sodium hydroxide*, 1 N.
*Ethyl acetate, water saturated*—Shake 150 ml of ethyl acetate with 15 ml of water for 3 minutes, and then allow the layers to separate.

TABLE III

ASSAY OF THREE SAMPLES OF CASCARA DRY EXTRACT BY THE METHODS
GIVEN IN APPENDIX II

| Sample | Laboratory | Aloins, as cascaro-side A, per cent. | Ratio ($E_{500}$ to $E_{440}$) | Cascarosides, as cascaro-side A, per cent. | Ratio ($E_{500}$ to $E_{440}$) | Total glycosides, per cent. |
|---|---|---|---|---|---|---|
| 1 | A | 6·48 | 1·98 | 3·62 | 2·03 | 10·10 |
|  |  | 6·57 | 1·98 | 3·68 | 1·99 | 10·25 |
|  | C | 8·83 | 1·86 | 4·55 | 1·92 | 13·38 |
|  |  | 6·95 | 1·92 | 5·99 | 1·45 | 12·94 |
|  | D | 8·68 | 2·02 | 4·53 | 1·92 | 13·21 |
|  |  | 8·84 | 1·98 | 4·10 | 1·94 | 12·94 |
|  | E | 8·90 | 1·93 | 4·60 | 2·00 | 13·50 |
|  |  | 8·80 | 1·94 | 4·70 | 2·09 | 13·50 |
|  | F | 8·75 | 2·00 | 4·63 | 1·90 | 13·38 |
|  |  | 8·67 | 1·98 | 4·56 | 2·01 | 13·23 |
|  | G | 8·20 | 2·05 | 4·30 | 2·05 | 12·50 |
|  | H | 9·52 | 1·80 | 4·18 | 2·01 | 13·70 |
|  |  | 9·42 | 1·85 | 4·28 | 1·86 | 13·70 |
|  | Mean  .. .. .. | 8·35 | 1·95 | 4·44 | 1·94 | 12·79 |
|  | Standard deviation  .. | 1·02 | 0·07 | 0·58 | 0·16 | 1·21 |
|  | Coefficient of variation, per cent.  .. .. | 12·22 | 3·77 | 13·07 | 8·28 | 9·45 |
| 2 | A | 5·97 | 2·01 | 4·65 | 1·93 | 10·62 |
|  |  | 6·00 | 2·05 | 4·54 | 2·18 | 10·54 |
|  | C | 7·57 | 1·86 | 6·10 | 2·08 | 13·67 |
|  |  | 7·10 | 1·94 | 6·17 | 2·04 | 13·27 |
|  | D | 7·92 | 2·16 | 6·54 | 2·09 | 14·46 |
|  |  | 8·30 | 1·94 | 5·70 | 1·92 | 14·00 |
|  | E | 9·04 | 1·73 | 5·76 | 1·98 | 14·80 |
|  |  | 8·96 | 1·74 | 5·62 | 2·01 | 14·58 |
|  | F | 7·87 | 2·03 | 6·08 | 2·12 | 13·95 |
|  |  | 8·03 | 1·99 | 6·49 | 2·11 | 14·52 |
|  | G | 7·50 | 2·05 | 5·70 | 2·11 | 13·20 |
|  | H | 8·74 | 1·88 | 5·64 | 2·06 | 14·38 |
|  |  | 8·72 | 1·88 | 5·59 | 2·08 | 14·31 |
|  | Mean  .. .. .. | 7·82 | 1·94 | 5·74 | 2·05 | 13·56 |
|  | Standard deviation  .. | 1·01 | 0·12 | 0·60 | 0·08 | 1·41 |
|  | Coefficient of variation, per cent.  .. .. | 12·86 | 6·42 | 10·45 | 3·70 | 10·40 |
| 3 (Spray) dried) | A | 5·30 | 1·92 | 9·50 | 2·05 | 14·80 |
|  |  | 5·14 | 1·91 | 9·48 | 2·04 | 14·62 |
|  | C | 5·56 | 1·87 | 9·08 | 2·07 | 14·64 |
|  |  | 5·56 | 1·93 | 9·08 | 2·07 | 14·64 |
|  | D | 5·10 | 1·81 | 9·02 | 2·13 | 14·12 |
|  |  | 4·76 | 1·89 | 9·64 | 2·09 | 14·40 |
|  | E | 4·92 | 1·70 | 8·58 | 2·02 | 13·50 |
|  |  | 4·80 | 1·70 | 9·50 | 1·92 | 14·30 |
|  | G | 4·76 | 1·80 | 8·71 | 1·99 | 13·47 |
|  |  | 4·87 | 1·84 | 8·99 | 2·16 | 13·86 |
|  | H | 5·47 | 1·69 | 9·13 | 2·00 | 14·60 |
|  |  | 5·51 | 1·64 | 8·79 | 2·07 | 14·30 |
|  |  | 5·11 | 1·65 | 8·94 | 2·10 | 14·05 |
|  | Mean  .. .. .. | 5·14 | 1·80 | 9·11 | 2·05 | 14·25 |
|  | Standard deviation  .. | 0·31 | 0·11 | 0·33 | 0·06 | 0·44 |
|  | Coefficient of variation, per cent.  .. .. | 6·04 | 6·00 | 3·65 | 3·04 | 3·06 |

PROCEDURE—

Weigh accurately $1 \pm 0.05$ g of sample, and add it, with stirring, to 100 ml of boiling water in a beaker. Continue boiling and stirring for 5 minutes. Cool, transfer the mixture to a 100-ml calibrated flask, dilute to the mark with water, shake the mixture well, and filter it through a Whatman No. 4 or other suitable filter-paper. By pipette, transfer 10·0 ml of the filtrate to a separating funnel, add 2 drops of 1 N hydrochloric acid, and extract with two 20-ml portions of carbon tetrachloride. Wash the combined carbon tetrachloride extracts with 5 ml of water, and return the washings to the aqueous layer. Discard the washed carbon tetrachloride extracts. Extract the aqueous layer with four 30-ml portions of freshly prepared water-saturated ethyl acetate, on each occasion allowing separation to take place until the solvent layer is clear. Combine the four ethyl acetate extracts, and reserve both layers for further work.

*Determination of aloins*—Transfer the combined ethyl acetate extracts to a suitable flask, distil off the solvent, and evaporate just to dryness. Dissolve the residue in 0·3 to 0·5 ml of methanol, rinse the solution out with warm water into a 50-ml calibrated flask, cool, and dilute to the mark with water. Transfer 20·0 ml of the solution to a 100-ml round-bottomed flask containing 1·2 g of anhydrous iron(III) chloride [or 2 ml of a 60 per cent. $m/V$ solution of anhydrous iron(III) chloride] and 12 ml of hydrochloric acid (36 per cent. $m/m$). Attach a water-cooled double-surface condenser to the flask, place the flask in a bath of continuously boiling water (so that the water level is above that of the liquid level in the flask), and heat it for 4 hours. Then set it aside to cool, transfer the solution to a separating funnel, and rinse out the flask successively with 3 to 4 ml of 1 N sodium hydroxide solution and 3 to 4 ml of water, adding these rinsings to the contents of the separating funnel.

Extract the contents of the separating funnel with three 30-ml portions of carbon tetrachloride. Wash the combined carbon tetrachloride layers with two 10-ml portions of water. Discard the washings, and dilute the carbon tetrachloride layer to 100·0 ml with the same solvent. Transfer 20·0 ml of this solution to a suitable flask, and evaporate carefully to dryness on a bath of boiling water. Dissolve the residue in 10·0 ml of 1 N sodium hydroxide solution, and within 5 minutes measure the extinction of this solution at 440 nm and at the maximum at about 500 nm in a 1-cm cell against 1 N sodium hydroxide solution.

Calculate the percentage of aloins present, as cascaroside A, on the assumption that $E_{1\text{cm}}^{1\%}$ at 500 nm of the red solution obtained from cascaroside A is 125. If the ratio of the extinction at 500 nm to that at 440 nm is less than 1·7, reject the result.

*Determination of cascarosides*—Dilute the aqueous layer reserved from the preliminary extraction to 50·0 ml with water. Treat 20·0 ml of this solution as described under *Determination of aloins*.

Calculate the percentage of cascarosides present, as cascaroside A, on the assumption that $E_{1\text{cm}}^{1\%}$ at 500 nm of the red solution obtained from cascaroside A is 125. If the ratio of the extinction at 500 nm to that at 440 nm is less than 1·8, reject the result.

## Appendix II

### RECOMMENDED METHOD FOR THE ASSAY OF CASCARA DRY EXTRACT

REAGENTS—

As described in Appendix I with the addition of the following reagent.
*Ethanol*, 70 *per cent*. V/V.

PROCEDURE—

Weigh accurately $0.5 \pm 0.05$ g of powdered extract and place it in a 100-ml calibrated flask with 80 ml of 70 per cent. ethanol. Shake the mixture occasionally, allow it to stand overnight, make the volume up to 100 ml with 70 per cent. ethanol, shake the mixture well, and filter it through a Whatman No. 4 filter-paper. By pipette, transfer 10·0 ml of the filtrate to a separating funnel, add 10 ml of water and 2 drops of 1 N hydrochloric acid, and extract with two 40-ml portions of carbon tetrachloride. Wash the combined carbon tetrachloride extracts with 5 ml of water, and return the washings to the aqueous layer. Discard the washed carbon tetrachloride extracts. Extract the aqueous layer with four 30-ml portions of freshly prepared water-saturated ethyl acetate, on each occasion allowing the solvent layer

to become clear before running off the aqueous phase.   Combine the four ethyl acetate extracts, and reserve both layers for further work.

Continue with the determination of aloins and cascarosides as described in Appendix I.

## REFERENCES

1.   Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry, *Analyst*, 1968, **93**, 749.
2.   "European Pharmacopoeia," Volume II, Maisonneuve S.A., Sainte Ruffine, France, 1971, p. 355.
3.   Fairbairn, J. W., and Simic, S., *J. Pharm. Pharmac.*, 1964, **16**, 450.

# Book Reviews

THIRD INTERNATIONAL CONGRESS OF ATOMIC ABSORPTION AND ATOMIC FLUORESCENCE SPECTRO-
METRY. PARIS, 27 SEPTEMBER–1 OCTOBER 1971. ORGANIZED BY LE GROUPEMENT POUR
L'AVANCEMENT DES MÉTHODES PHYSIQUES D'ANALYSE (GAMS), LE COMMISSARIAT À
L'ENERGIE ATOMIQUE, LA FACULTÉ DE PHARMACIE DE PARIS. 69 PAPERS PRESENTED
AT THE CONGRESS. Edited by M. PINTA. Volume 1, Pp. xviii + 398; Volume 2, Pp. xviii
+ 399–924. London: Adam Hilger. 1973. Price £8 the set.

With the exception of the plenary lectures, most of the papers presented at the Third Inter-
national Congress on Atomic Fluorescence and Absorption Spectrometry (ICAFAS), held in Paris
in September, 1971, appear in these two official volumes. The nine plenary lectures were pub-
lished in the journal *Méthodes Physiques d'Analyse* (GAMS, Paris) in a special issue in September,
1971, and in the March, 1972, issue (Vol. 8, No. 1).

Volume 1 begins with the Congress opening address by Professor M. L. Girard, in which the
development of atomic-absorption and atomic-fluorescence spectroscopy are discussed, and the
two previous Conferences held in Prague and Sheffield, respectively, are briefly referred to. There-
after, the subject headings of this volume (number of papers in parentheses) are as follows: Theory
and Methodology (10), Apparatus (10), Atomic Fluorescence (3) and Rocks, Soils and Minerals (9).
Volume 2 has about the same number of main headings and contributions, *viz.*, Water, Agriculture
and Related Subjects (11), Biology (10), Metals (5) and Miscellaneous (11); it also includes a
cumulative Author Index. The section covering Metals deals with, for example, the determination
of trace amounts of platinum metals, versatile applications of atomic-absorption spectroscopy in
the steelworks industry, typified by the analysis of raw materials, the determination of atmospheric
pollutants and trace amounts of aluminium in silicon-bearing steels. The latter and the deter-
mination of iron in fruit drinks are both described in detail in this section. Incidentally, the paper
on The Application of "Difference Atomic Absorption" to the Accurate Determination of Major
Concentrations, in the Biology section, should, more appropriately, appear under Metals.

Of the 69 papers contained in this two-volume lithographic publication, one is in German,
the remainder being about equally divided between the French and English languages and, with
the exception of the Presidential Address, which is in French, each of the technical contributions
has summaries in each of these three languages.

These two companion volumes present a panoramic view of the status of atomic-absorption
and atomic-fluorescence (to a lesser extent flame-emission) spectroscopy up to the time of the
Congress but, despite the commendable efforts made by those responsible for the early release
of these papers in book form, it is almost inevitable that information in these rapidly advancing
fields soon becomes out-dated, even though only about 2 years have elapsed since the Congress
was held.                                                                    W. T. ELWELL

ION-SELECTIVE ELECTRODES. SYMPOSIUM HELD AT MÁTRAFÜRED, HUNGARY, 23–25 OCTOBER,
1972. Edited by Professor E. PUNGOR. Technical Editor I. BUZÁS. Pp. 283. Budapest:
Akadémiai Kiadó. 1973. Price £3·50.

This book, produced by offset lithography from double-spaced, typewritten copy in English, is
based on the Proceedings of the Symposium on Ion-selective Electrodes held at Mátrafüred in
October, 1972. The first two thirds of the book is devoted to the seven invited plenary lectures, which
were centred mainly on fundamental aspects of electrode design, behaviour and applications.
One of these lectures was directed to the applications of ion-selective electrodes in continuous
analysis. The remaining third of the book is given to the ten contributed lectures followed by a
short summary of the symposium discussion. Not unnaturally, most of the contributed lectures
were based on silicone rubber matrix membrane electrodes, although some attention was also
given to Crytur solid-state electrodes as well as to liquid membrane and coated wire systems.

Regarding the analytical viewpoint, appropriate conditions for using the electrodes are
described, but the range of illustrative applications is necessarily limited. Thus, although some
sections will be helpful to the analyst, the volume, being a record of symposium proceedings, is
likely to be of more specialised interest to those in the ion-selective electrode field.

                                                                    J. D. R. THOMAS

SELECTED ANNUAL REVIEWS OF THE ANALYTICAL SCIENCES. Edited by L. S. BARK. Volume 1. Pp. vi + 269. 1971. Volume 2. Pp. vi + 149. 1972. London: Society for Analytical Chemistry. Price £5 per volume.

This review is concerned with the first two volumes of a series of annual reviews to be published by the Society for Analytical Chemistry. The purpose of the series is to assist scientists with an interest in analysis to keep abreast of developments in the subjects covered. Each volume contains several comprehensive discussions of significant developments in a selected subject area during the preceding four or five years.

The contents of Volume 1 (1971) are as follows: Molecular-sieve Chromatography, by D. M. W. Anderson, I. C. M. Dea and A. Hendrie; Photoluminescence and Chemiluminescence in Inorganic Analysis, by L. S. Bark and P. R. Wood; Recent Developments in Activation Analysis, by T.B. Pierce; Atomic-absorption Spectroscopy, by P. Platt; and Catalytic Methods in Analytical Chemistry, by G. Svehla.

Volume 2 (1972) contains: The Techniques and Theory of Thermal Analysis Applied to Studies on Inorganic Materials with Particular Reference to Dehydration and Single Oxide Systems, by D. Dollimore; Developments in Ion Exchange, by F. Vernon; and Thermometric and Enthalpimetric Titrimetry, by L. S. Bark, P. Bate and J. K. Grime.

The treatment of the subject matter of this series differs significantly from that of other reviews with which most analytical chemists are familiar. The subjects are more specific than general, and the authors have been given free rein to select material in order to present a state-of-the-art coverage in a systematic manner. Sufficient discussion is included to make the reviews self-contained in most instances. The reader will, of course, consult the references, as well as those of other bibliographic reviews for technical details.

The reviews contained in the present volumes are well presented and should prove to be very valuable to analytical chemists working in the subject fields or in related areas or for those who require only a current awareness of progress in analytical science. It is hoped that the series will continue and expand to hasten the coverage of the broad field of modern analytical chemistry.

JOHN K. TAYLOR

ATLAS OF SPECTRAL DATA AND PHYSICAL CONSTANTS FOR ORGANIC COMPOUNDS. Edited by JEANNETTE G. GRASSELLI. Pp. xii + 1747. Cleveland, Ohio: The Chemical Rubber Co. 1973. Price £50.

It is now generally recognised that the vast amount of available scientific data can be managed only by the employment of sophisticated computerised information storage and retrieval systems. The success of such systems, however, depends greatly on the quality and type of data stored; the production of this Atlas represents the first step towards the creation of a data-bank of sufficient quality to act as the basis of a computerised system for the chemical literature. In order to be of maximum utility, a base of "hard" data of sufficient magnitude and potential was required. It was decided that the first section of the data-bank to be compiled would consist of the spectral data and physical properties for approximately 8000 compounds presented in printed format in the present volume. For the first time, comprehensive data including name, synonyms, empirical and structural formulae, Chemical Abstracts Registry Number, Wiswesser line notation, boiling-point, melting-point, density, refractive index, specific rotation, solubilities, relative molecular mass, literature references, and infrared, ultraviolet, nuclear magnetic resonance and mass spectral data are all combined for these compounds into a single volume for desk-top use.

The volume is well laid out and the material is presented concisely. The spectral data are coded with emphasis on the selection of values characteristic of compound structure, not necessarily striving for a complete representation of the original spectra. The physical constants given for each compound are abridged from the Tables of Physical Constants of Organic Compounds section in the Chemical Rubber Company's "Handbook of Chemistry and Physics." It is planned to review and update these values in each edition of the Atlas. The inclusion of the Wiswesser line notation for each compound is an innovation; for those willing to expend a small effort in order to become familiar with this concise and unambiguous representation of chemical structure, however, it is a powerful aid in compound searching and identification. The Atlas can be used to obtain information, including spectral data, on the compounds covered or, conversely, to identify an unknown compound from some characteristic physical constant or from spectral data. A set of comprehensive indexes is given and their application is explained in the foreword to each.

The publication of this Atlas represents the culmination of several years of dedicated effort on behalf of the Editor and her associates. It is a staggering production, even if judged only from its physical proportions. It comprises almost 1800 oversized pages and contains well over one million items of evaluated data. The expertise and courage required in order to organise and complete the production of the Atlas is indeed impressive. As part of an immense design to create a computerised data-bank of virtually unlimited capability for information storage and retrieval, this volume should certainly be made available in library facilities as a first edition and cornerstone of the system.                                                    G. F. KIRKBRIGHT

ANNUAL REPORTS ON NMR SPECTROSCOPY. Volume 5a. Edited by E. F. MOONEY. Pp. xii + 696. London and New York: Academic Press. 1972. Price £13.

Volume 5 in this well established series is divided into two parts in an effort to keep the price down. Volume 5b deals with the nuclear magnetic resonance parameters of phosphorus compounds.

The present volume (5a) starts with a general review of proton magnetic resonance by T. N. Huckerby, and is a critical survey of the 1970 literature. There follow chapters on fluorine-19 nuclear magnetic resonance spectroscopy (R. Fields) and on nuclear magnetic resonance spectroscopy in the study of carbohydrates and related compounds (T. D. Inch); the former is a somewhat uncritical review of the literature for 1970, the latter an updating, covering 1968–70. Both chapters will have some general appeal although they are more for the specialist. A chapter on heteronuclear double resonance (W. McFarlane) updates an earlier review (1968), as does a chapter on nitrogen nuclear magnetic resonance spectroscopy (M. Witanowski and G. A. Webb). Both will be useful but it is a pity that virtually no literature later than 1970 is quoted.

There follows a chapter on nuclear magnetic resonance spectroscopy in liquids containing compounds of aluminium and gallium (J. W. Akitt), a specialist topic but welcome because it reviews all the literature to 1970 and includes some references for 1971 and 1972. The final chapter deals with the application of Fourier transformation to high-resolution nuclear magnetic resonance spectroscopy (D. G. Gillies and D. Shaw). Activity in this last field is high and is being sustained by the application of the technique to the carbon-13 nucleus. It is a timely review but it does meet with some competition.

This is a well produced book, but at £13 is probably suited only to library purchase. It is a pity that, where relevant, the literature was not surveyed to a later date. The book would be even more useful if it had been produced earlier.                              W. I. STEPHEN

# Errata

JUNE (1973) ISSUE, p. 447, line 26. *For* "100 ml" *read* "1000 ml."
SEPTEMBER (1973) ISSUE, p. 682, Table VII, 4 lines from the bottom. *For* "170·6" *read* "70·6."

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## A Spectrophotometric Method for the Micro-determination of Piperonyl Butoxide in the Presence of Pyrethrins

A rapid spectrophotometric method has been developed for the micro-determination of piperonyl butoxide in the presence of a wide range of pyrethrins. A solution of the mixture of piperonyl butoxide and pyrethrins in a low-boiling fraction of light petroleum is evaporated on a water-bath until only a small portion of the solvent remains. The final traces of solvent are removed at 50 °C and, to the residue containing piperonyl butoxide and pyrethrins, 18 per cent. $m/V$ nitric acid is added in order to convert the piperonyl butoxide content into a soluble yellow-coloured compound. The quantitative colour reaction has a maximum absorption at 370 nm, and the method is applicable to the residues in the range 4 to 40 $\mu g$ ml$^{-1}$ of piperonyl butoxide. The method is also applicable to the residues of formulated products extracted from grains and paper coatings.

**H. M. BHAVNAGARY and S. M. AHMED**
Central Food Technological Research Institute, Mysore-2A, India.

## The Determination of Chlorhydroxyquinoline in Medicated Pig Feeds

A method has been developed for the determination of chlorhydroxyquinoline (Halquinol) in medicated pig feeds. Because of the interference by feed constituents in simple spectrophotometric and polarographic assay procedures, a spectrofluorimetric procedure is recommended. Spectrofluorimetric measurements are taken in a methanolic solvent containing 5 per cent. of chloroform, and the fluorescence of chlorhydroxyquinoline, as its magnesium chelate, is measured at 500 nm, with an excitation wavelength of 402 nm. Cyanide is used to suppress interference from copper and zinc salts that are commonly added to these feeds. The procedure is not affected by the presence of other feed additives, such as dimetridazole and arsanilic acid.

**J. E. FAIRBROTHER and W. F. HEYES**
Squibb International Development Laboratory, Moreton, Wirral, Cheshire.

## Solvent Extraction of Copper(II) and Zinc(II) with 1,5-Diphenylcarbazone

The extraction characteristics of 1,5-diphenylcarbazone and its complexes with the bivalent metal ions copper(II) and zinc(II) in an isobutyl methyl ketone - water system have been studied and the extraction curves of these metal complexes have also been obtained. The copper(II) complex is extracted from a more acidic solution than is the zinc(II) complex. The extraction equilibria have been examined and the extraction constants determined. The spectral properties of the complexes have also been determined and the application of the reagent to the determination of copper and zinc is suggested.

**HISAHIKO EINAGA and HAJIME ISHII**
National Institute for Researches in Inorganic Materials, Niihari-gun, Ibaraki-ken, Japan.

## A Solvent-saving Extraction - Evaporation Apparatus Developed for Residue Analysis of Pesticides

An apparatus that simultaneously allows Soxhlet extraction of pesticides and concentration of the resulting extract has been designed. The evaporated solvent is refluxed into the Soxhlet flask where it is utilised again for the extraction. The major advantages of this cyclic extraction - evaporation system are that redistillation of solvents prior to extraction can be omitted, that manual work is reduced, as the solvent evaporation process occurs simultaneously with the extraction of the sample, and that excess of solvent is not wasted but re-utilised for extraction, which is of interest from the economic and environmental contamination points of view.

**G. VOSS and W. BLASS**
Agrochemical Division, CIBA-Geigy Ltd., Basle, Switzerland.

## Fractionation and Identification of Commercial Hydrocolloid Stabilising Agents

### Part II. Identification of the Components of Guar Gum - Locust Bean Gum and of Pectinate - Gum Tragacanth Mixtures

The limitations of a method for the identification of mixtures of stabilising agents described previously have been overcome.   The method now facilitates positive identification of pectinate and tragacanth in admixture and of guar gum and locust bean gum in admixture.

**R. G. MORLEY, G. O. PHILLIPS, D. M. POWER**
Department of Chemistry and Applied Chemistry, University of Salford, Salford, Lancashire, M5 4WT.

**and R. E. MORGAN**
The Medical Center, University of Alabama, Birmingham, Alabama, U.S.A.

## Determination of Nicotinamide in Some Injections of B-complex Vitamins by Thin-layer Chromatography

Nicotinamide in some injections of B-complex vitamins can be separated on a thin layer of silica gel G by using a solvent mixture of ethanol (98 per cent.) - chloroform $(25 + 60 \ V/V)$, eluted from the scraped off area with $0 \cdot 1$ N hydrochloric acid and the solution measured spectrophotometrically at 261 nm.   Results obtained by use of the proposed method are compared with those obtained with the ammonia distillation method of the British Pharmacopoeia 1968.

**SAAD A. ISMAIEL and DAWOUD A. YASSA**
Research  Department, Société Misr pour l'Industrie Pharmaceutique, 92 El-Mataria Street, Post El-Zeitoun, Cairo, Egypt.

## Determination of Balsamic Acids and Esters by Gas - Liquid Chromatography

A gas - liquid chromatographic method for the determination of balsamic acids and esters in crude drugs is described.   Methylation of free acids is followed by a single-stage separation and quantitative determination of methyl and benzyl esters of benzoic and cinnamic acids.   Results are presented for the column parameters and reproducibility of the method.   The analysis of a commercial sample of tolu balsam is reported; the interpretation of the results may offer additional information to that obtained from official standards.

**K. J. HARKISS and P. A. LINLEY**
Postgraduate School of Studies in Pharmacy, University of Bradford, Bradford, BD7 1DP.

## Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

### Part III.  The Determination of Geraniol in Oils of Citronella
Report prepared by the Essential Oils Sub-Committee.

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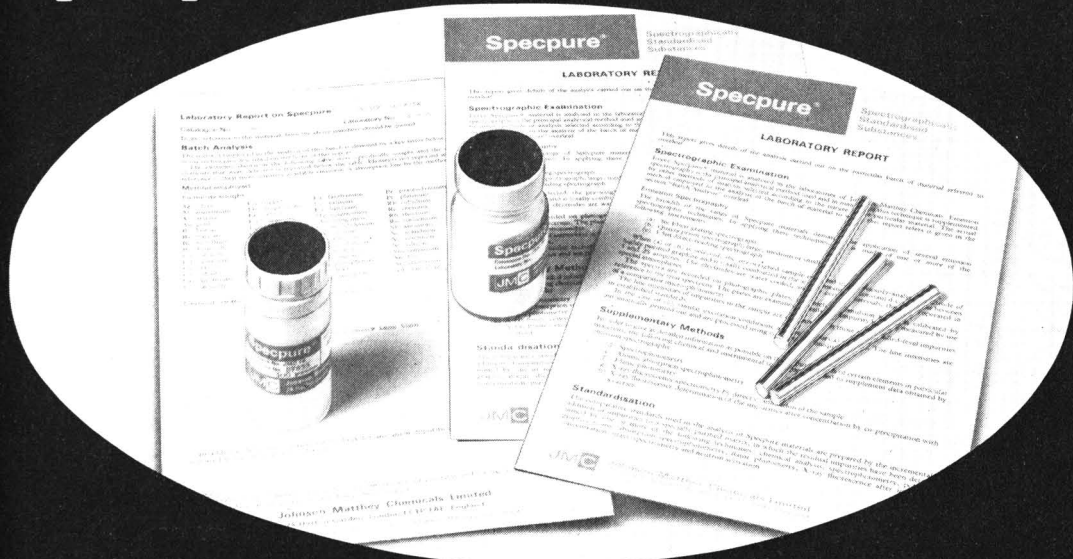


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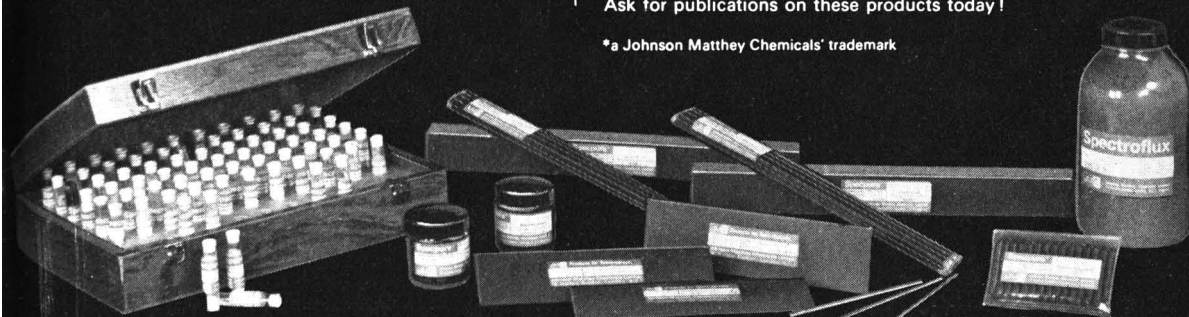


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