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Established as a medium for the rapid publication of papers dealing with all aspects of analytical chemistry, *Talanta* is the natural vehicle for the international communication of progress in this field. As an international journal, its papers are expected to be of a very high standard and to make definite contributions to the subject: they must be new publications. Papers may be written in English, French, or German; all papers have abstracts in these three languages and also in Russian. Special importance is attached to work dealing with the principles of analytical chemistry in which experimental material is critically evaluated, and to similar fundamental studies. Reviews in rapidly expanding fields or of hitherto widely scattered material are considered for publication, but should be critical. Original papers, short communications and reviews are refereed in the normal manner. Preliminary communications are refereed urgently and accorded priority in publication. Correspondence of interest to analytical chemists is welcomed by the Editor-in Chief, at whose discretion it is published. A new feature is Annotations, which are critical commentaries on some aspect of analytical chemistry and deal with topics such as sources of error, or the scope and limitations of methods and techniques; these commentaries are refereed.

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TALANTA REVIEW*

FLUORESCENCE ANALYSIS IN AIR POLLUTION RESEARCH

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Summary—Luminescence phenomena are of value in the analysis of air pollutants. The problems arising in the use of excitation and emission spectra under various conditions are discussed. Phenomena such as solvent, pH, and photochemical effects are shown to play an important role in the fluorimetric analysis of air pollutants. Many of the fluorimetric methods used in the trace analysis of organic airborne particulates involve factors such as direct measurement of the separated pollutant on a chromatogram or pherogram, quenching phenomena, scanning, excimer formation, charge-transfer fluorescence, sensitized fluorescence, and photo-oxidation on adsorbent or in solution. In addition, fluorescence assay methods are discussed in terms of selectivity, sensitivity, speed, simplicity, accuracy, precision, interferences, and the relation between concentration and fluorescence intensity.

LUMINESCENCE phenomena have been applied to air pollution studies in only a few laboratories. However, the little work that has been done indicates that further developments in fluorescence analysis should prove invaluable in studies concerning our environment, especially because of the high selectivity and sensitivity inherent in such analysis.

If investigators of the human environment are familiarized with some of the advances in this field, they may be encouraged to explore and to exploit fluorimetry and phosphorimetry for better understanding and more sensible control of our environment.

Results of experiments will be considered from the viewpoints of utility of the analytical procedures, understanding of the problems that arise in luminescence analysis, and potentiality in future applications to air pollution research.

FLUORESCENCE IN SOLUTION

Since most analyses are performed on solutions, solvent properties, as well as solvent–solute and solute–solute interactions, play an important role in fluorimetry.

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Excitation spectra

Comparison with absorption spectra. Under ideal conditions the excitation and absorption spectra of a compound in a solution should be parallel to each other at all wavelengths provided that (*a*) the quantum yield is independent of the exciting wavelength, (*b*) the quantum intensity of the exciting light is kept constant as the wavelength is varied, (*c*) the solution (solid or liquid) is sufficiently dilute for its absorbance to be small at the wavelength maxima and (*d*) only one fluorescent solute

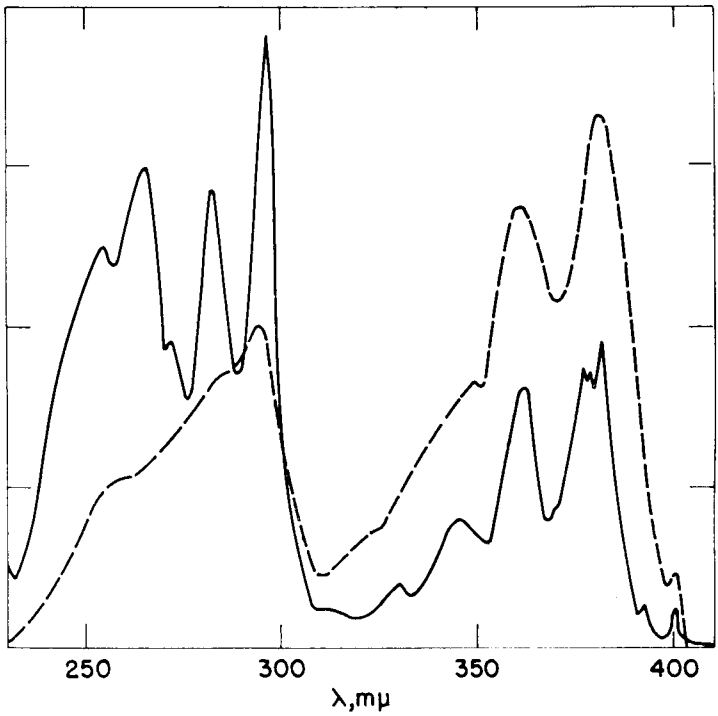


FIG. 1.—Benzo[*a*]pyrene in pentane.
(———) Absorption spectrum; (– – – –) excitation spectrum at a fluorescence wavelength of 403 nm.[2] (Reprinted from *Intern. J. Air Pollution* 1960, **2**, 253.)

is present.[1] This means that the shape of the fluorescence excitation spectrum of a solid or liquid solution containing one fluorescent solute should be independent of the wavelength of light at which the emission monochromator has been set. When new excitation spectral bands or different relative intensities of such bands are obtained with the emission monochromator set at different emission wavelength maxima, then the solution probably contains at least two fluorescent species.

The excitation spectra obtained with most commercial spectrophotofluorimeters are distorted versions of the true spectra and vary in position of the wavelength maximum from one instrument to another. Since the xenon arc gives a continous spectrum over the ultraviolet and visible regions, it is used as the source of light energy. However, its intensity varies with wavelength, diminishing at shorter wavelengths. Consequently, instrumental excitation maxima appear at longer wavelengths than do the true maxima.

Figure 1 compares the excitation and absorption spectra obtained for a $10^{-5}M$

pentane solution of benzo[*a*]pyrene.[2] The excitation spectrum has less fine structure than the absorption spectrum, although the relative intensities of the peaks in the 300–400 nm region are approximately the same in both types of spectra. However, at wavelengths below 300 nm the extreme distortion of the excitation spectrum would be a serious interference in any characterization study of air pollutants.

One advantage of the excitation spectrum over the absorption spectrum is its greater selectivity, since fewer compounds fluoresce than absorb light. In addition, excitation can be measured in the presence of strongly absorbing organic compounds if the solution is dilute enough. Because of its selectivity and sensitivity, the excitation spectrum provides another means of measuring the absorption spectra of nanogram to microgram amounts of air pollutants, amounts for which absorption spectra cannot be obtained with commercial instruments. In an analysis of a mixture of absorbing organic compounds, the excitation spectrum of a fluorescing compound can often be obtained directly (if the emission monochromator is set at the proper wavelength) under conditions in which the absorption spectrum of the compound would be hidden by the background absorption of the other compounds. Examples of the use of this technique are detection of traces of mineral oils[3] and detection of oil mist in air at concentrations <1 μg/l.[4]

Obviously, readily-obtained, corrected excitation spectra would be invaluable in trace analysis since they could be compared directly with absorption spectra. Instruments to provide such spectra are highly expensive, and even then, other factors, as will be shown, could preclude such "perfect" spectra. For most trace analysis, corrected spectra are unnecessary.

Absorption spectra do show some advantages over excitation spectra obtained with most commercial instruments. The absorption spectra of weakly polar, unsaturated organic compounds contain many sharp, intense vibrational bands of various electronic transitions, bands which are absent from the excitation spectra. Thus, the longer wavelength absorption bands are useful for characterization and assay purposes since they show up through background material. This type of analysis cannot be used for the more polar compounds since their bands are broader and thus blend into the background more readily. In addition, absorption spectra do not show the distortions due to quenching, concentration, and other effects which can alter excitation spectra so drastically. The positions of the wavelength maxima are also more definite and more reproducible than are the positions of the excitation spectra maxima.

The intensity, selectivity, and completeness of an excitation spectrum obtained from the solution of a fluorescent compound depends to a large extent on the emission wavelength at which the excitation spectrum is obtained, Fig. 2.[5] The closer the emission monochromator wavelength is set to the excitation spectrum, the more interference occurs from scatter.

Concentration or inner-filter effects. The intensities of the fluorescence excitation and emission spectra are proportional to the concentration of the fluorescent compound only in dilute solution. At higher concentrations the intensity falls off, and two widely different concentrations can give the same intensity reading. Consequently, several dilutions of the test solution should be examined.

Under ideal conditions, every molecule within the light path is illuminated equally, and the emitted radiation is not affected by the presence of solute molecules. Should

either or both of these conditions not be obeyed, an inner-filter effect occurs, *i.e.*, fluorescence-emission or -exciting radiation is absorbed by species in solution or on a chromatogram or pherogram. An example is shown in Fig. 3. The fluorescence excitation spectrum of $10^{-6}M$ perylene shows little distortion. With $10^{-5}M$ solution, the long-wavelength band at 430 nm is depressed more than that at 405 nm. The $10^{-4}M$ solution shows the effect of excessive absorption of exciting light. The bands

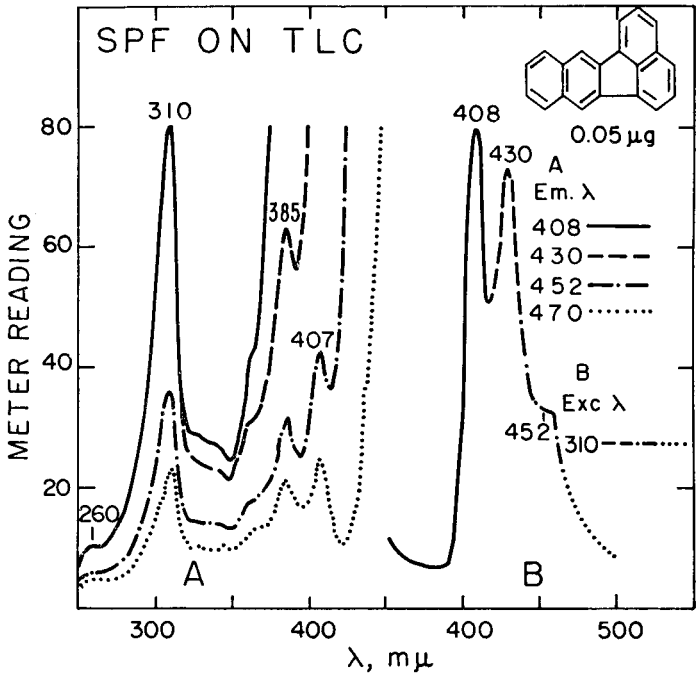


FIG. 2.—Fluorimetric excitation (A) and emission (B) spectra of benzo[*k*]fluoranthene on an alumina–cellulose acetate (2:1) thin-layer plate.
SPF on TLC = direct spectrophotofluorimetric examination of the thin-layer plate.
Exc. λ(em. λ) = excitation (emission) wavelength at which the emission (excitation) spectrum was obtained.[5]

at 350 and 450 nm are reproducible but spurious. The spectrum from 385 to 430 nm is an inversion of the spectrum of the more dilute solution. Thus, the minima at 385, 405 and 430 nm for the $10^{-4}M$ solution are inversions of the shoulder at 385 nm and the maxima at 405 and 430 nm.

Emission spectra

Mirror image rule. For many organic compounds, the fluorescence emission spectrum is a mirror image of the absorption spectrum, and the position of the long-wavelength band of the absorption spectrum almost coincides with the position of the short-wavelength band of the emission spectrum. Since the fine structure in an excitation or absorption spectrum is derived from transitions involving the vibrationally excited states of the lowest excited electronic state and the fine structure in a fluorescence emission spectrum is derived from transitions involving the vibrationally

excited states of the ground state, the nuclear configuration of the molecule is similar in both states if the mirror image rule is obeyed.

The lack of such a relation between the absorption and emission spectra of a compound indicates a large change in the spatial configuration of the molecule upon electronic excitation.[6]

If the lowest wavelength emission band (usually at longer wavelength than the absorption bands) is far removed from the longest wavelength excitation band, then the solution may contain at least two species.

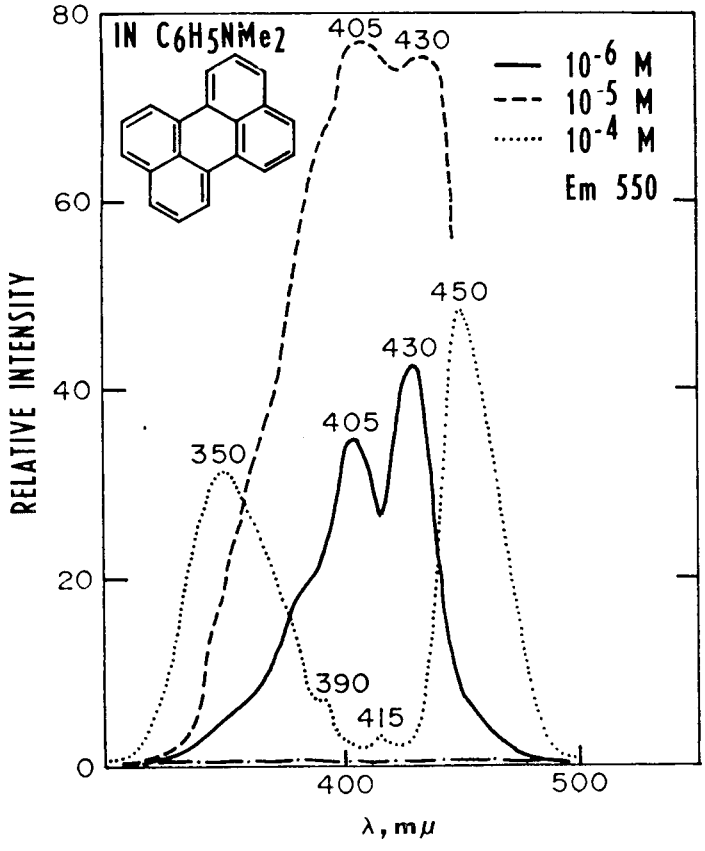


FIG. 3.—Effect of concentration of perylene on the fluorescence excitation spectra in *N,N*-dimethylaniline with the emission monochromator set at 550 nm.

Concentration or inner-filter effects. Another important inner-filter effect interfering in trace analysis is that arising from absorption of the fluorescent light by the solution. An excessive concentration of the fluorescing solute can result in self-absorption of the emitted light. The presence of high concentrations of other compounds can cause the inner-filter effect by distortion of the emission spectrum. These two effects mainly decrease the intensity of the short-wavelength emission band relative to that of the other emission bands.

Selectivity. One of the great advantages of spectrophotofluorimetry is that besides the excitation spectra, the emission spectra give greater sensitivity and selectivity than are possible with absorption spectrophotometry. For example, the

emission spectrum of benzo[*a*]pyrene in sulphuric acid has been obtained in the presence of fifty aromatic and heterocyclic compounds, including benzo[*k*]fluoranthene, benzfluorenes, fluoranthene, benzo[*e*]pyrene, benzo[*ghi*]perylene, perylene and anthanthrene.

Standards. An absolute fluorescence intensity analogous to molar absorptivity is not obtained with commercial instrumentation. Consequently, measurements are made by reference to some appropriately chosen standard. 4-Dimethylamino-4′-nitrostilbene should be especially valuable since its emission spectrum changes drastically with the polarity of the solvent. Quinine is the standard most often used, even though its fluorescence characteristics "are the most unusual so far observed for any compound in solution."[7] Thus, the quantum yield of quinine is under dispute, the fluorescence probably arises from more than one excited state, the quantum efficiency is dependent on the excitation wavelength, the emission is dependent on the wavelength of the exciting light, and the excitation and absorption spectra do not coincide.

Anti-Stokes fluorescence. The emission spectra maxima are usually at longer wavelength than any excitation maximum. When an emission band is at shorter wavelength than the long-wavelength excitation band, then usually at least two fluorescent compounds are present.

Anti-Stokes fluorescence can, however, be derived from delayed fluorescence.[8] This arises when the donor singlet is at lower energy than that of the acceptor, as in the delayed fluorescence of trace amounts of anthracene in impure pyrene containing trace amounts of benz[*a*]anthracene and selectively sensitized by Acridine Orange. Thus, excitation at the donor's (Acridine Orange) excitation wavelength maximum of 436 nm gives the delayed fluorescence emission bands of anthracene at 380, 400, 420 and 445 nm. Parker's technique of selectively sensitized, anti-Stokes, delayed fluorescence may be of value for the direct analysis of some complex mixtures, with a minimum of separation.

An example of anti-Stokes excitation has been reported for phenanthrene.[9] In solution a small proportion of the molecules are thermally activated to an upper vibrational level; the transitions to the excited state require correspondingly less energy. Consequently, new absorption wavelengths are present at longer wavelengths than usual, and the longest of these is at longer wavelength than the shortest wavelength emission band. Thus, excitation at 366 nm gives an emission band at 358 nm.

Intensity

Solvent effects. Probably the most interesting and most troublesome aspect of fluorescence analysis is the remarkable change in intensity with change in solvent composition. Sulphanilamide, which has excitation wavelength maximum varying from 270 nm in isobutanol to 305 nm in toluene, has an emission wavelength maximum at 350 nm for all solvents, but the relative intensity varies from 0 to over 100.[10] A 1000-fold change in intensity with solvent change is usual for fluorescent molecules, so for each compound some solvent systems can enhance the fluorescence intensity and other solvents can decrease or quench the fluorescence.

This drastic change in fluorescence intensity has to be reckoned with in air-pollution trace analysis. The ideal solvent system in fluorescence trace analysis

is one in which the test substance has maximum fluorescence but the other compounds present have minimal fluorescence.

Structural effects. In addition to the solvent, the structure of the test substance can affect the intensity. A molecule which has absorbed energy can dissipate it through a radiationless process, can transfer most of it by intersystem crossover to the excited triplet state, or can emit it as radiation (fluorescence). The relative contributions of these various competitive processes depend to a large extent on the structure of a compound, even for related substances such as polynuclear aromatic hydrocarbons, see Table I.[2]

TABLE I.—COMPARISON OF FLUORESCENCE INTENSITIES OF POLYNUCLEAR HYDROCARBONS IN PENTANE

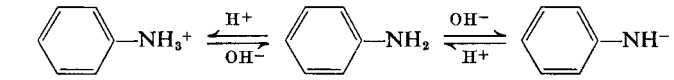
Compound	Wavelength maxima, *nm*		K_Q*
	Excitation	Fluorescence	
Quinine			1·0
Anthanthrene	420	430	45
Naphtho[2,3-*a*]pyrene	457	458	~33
Perylene	430	438	32
Dibenzo[*b*,*k*]chrysene	308	428	17†
7-Methyldibenzo[*a*,*h*]pyrene	460	467	13†
Benzo[*k*]fluoranthene	302	400	13
Benzo[*a*]pyrene	381	403	6
1,4-Diphenylbutadiene	328	370	6
p-Terphenyl	284	338	6
Benzo[*k*,*l*]xanthene	363	418	5
11*H*-Benzo[*a*]fluorene	317	340	4
11*H*-Benzo[*b*]fluorene	312	340	4
7*H*-Benzo[*c*]fluorene	334	337	4
Benzo[*b*]naphtho[2,3-*d*]furan	320	350	4
Anthracene	350	398	3
Benzo[*b*]chrysene	283	398	2
9-Methylanthracene	382	410	2
3-Methylcholanthrene	297	392	2
Benzo[*b*]fluoranthene	300	428	2
Dibenzo[*a*,*e*]pyrene	370	401	2
Tribenzo[*a*,*e*,*i*]pyrene	384	448	2
Dibenz[*a*,*h*]anthracene	292	394	1
Fluorene	300	321	1
Benz[*a*]anthracene	284	382	0·9
7,12-Dimethylbenz[*a*]anthracene	293	427	0·8
Fluoranthene	354	464	0·8
Dibenz[*a*,*j*]anthracene	300	410	0·7
Dibenz[*a*,*c*]anthracene	280	381	0·7
Picene	281	398	0·7
4-Methylpyrene	338	386	0·7
o-Phenylenepyrene	360	506	0·6
1-Methylpyrene	336	394	0·6
Chrysene	264	381	0·6
Coronene	337	450	0·6
Pyrene	330	382	0·5
3-Methylphenanthrene	292	368	0·5
Benzo[*e*]pyrene	329	389	0·4
Benzo[*g*,*h*,*i*]perylene	280	419	0·4
Acenaphthene	291	341	0·4
Phenanthrene	252	362	0·2
2-Methylphenanthrene	257	357	0·2
4-Cyclopenta[*d*,*e*,*f*]phenanthrene	294	362	0·2
Triphenylene	287	357	0·1
5,12-Dihydronaphthacene	282	340	0·1

* Intensity relative to quinone. † In chloroform.

Since rigid coplanar structures are conducive to intense fluorescence, the introduction of steric strain in a molecule can have an adverse effect on its fluorescence. If part of a chromophoric system is able to twist and turn, the molecule is usually non-fluorescent in water and in organic solvents. However, if the molecule is rigidified by dissolution in viscous media, by binding to large polymers in solution, by solidification in crystalline form, or by binding to an adsorbent, this tends to retard intramolecular twisting and to encourage fluorescence. For example, some triphenylmethane dyes are non-fluorescent in aqueous or organic solvents but become fluorescent in viscous media (glycerol) or by binding to large polymers in solution.[11,12] Formaldehyde forms sterically-hindered 2,6-dimethyl-3,5-dibenzoyl-1,4-dihydropyridine on reaction with 1-phenyl-1,3-butanedione,[13] this compound is non-fluorescent in solution, but becomes fluorescent when rigidified in aqueous alcohol at liquid-nitrogen temperatures or in the crystalline state.

pH Effects

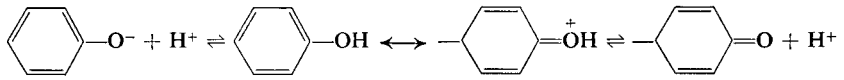
Change in alkalinity or acidity of a solution can have a profound effect on the fluorescence of a compound, especially if the compound can undergo ionization. Knowledge of fluorescence wavelength and/or intensity changes is indispensable in the development of methods of analysis for trace contaminants. Thus, for example, aniline is present at pH < 2 as the anilinium cation, from pH 7 to 12 as the neutral species, and above pH 13 mainly as the anion:[14]



Non-fluorescent F 290/345† Non-fluorescent

Formation of the anion is suggested by the fact that at high pH *N*-methylaniline is non-fluorescent but *N*,*N*-dimethylaniline is fluorescent. On the other hand, with some of the polynuclear aromatic amines, fluorescence is found for the anionic and cationic salts as well as for the neutral amine, *e.g.*, 8-aminofluoranthene.[15]

Excited state dissociation. The various acids, neutral and cationic, fall into two main classes. Members of one group are stronger acids in the excited singlet state, *i.e.*, $pK_a^* < pK_a$. These compounds lose a proton more readily in the excited state since the O, N, and S atoms have decreased electron densities in the excited state, as shown for phenol.[16]



pK_a 10·02 pK_a^* 5·7 ± 0·7

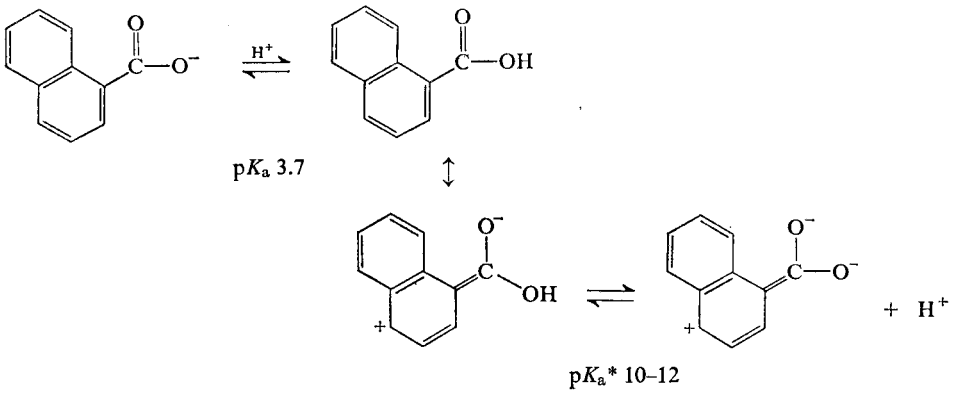
The structures on the left are important contributors to ground state ionization, and the structures on the right to excited state ionization.

Acids belonging to this group include ArOH, ArNHR, $ArNH_2$, ArR_2N^+H, and ArSH.

Members of the second main group are weaker acids in the excited singlet state *i.e.*, $pK_a^* > pK_a$. These compounds lose a proton more readily in the ground state

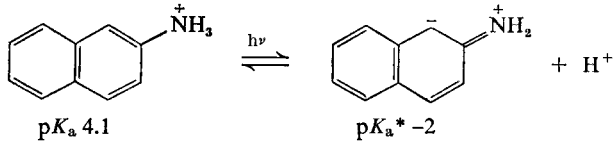
† *I.e.*, fluoresces with excitation maximum at 290 nm and emission maximum at 345 nm.

since the O, N, and S atoms have an increased electron density in the excited state, as shown for 1-naphthoic acid.[17] Acids belonging to this group include $ArCO_2H$,



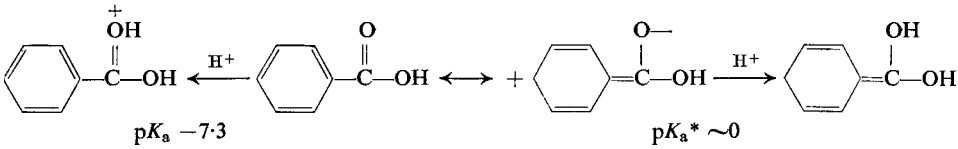
$ArC(R)=\overset{+}{O}H$ (R = H, alkyl, aryl or OH), and $\rangle N^+—H$.

Aromatic amines are weaker bases in the excited state because of the decreased electron density of the N atom in the excited state, as shown for 2-naphthylamine.[18]



Because of this large difference in basicity, absorption is by $ArNH_3^+$ between pH −2 and +4, while emission is from $ArNH_2$. The zwitterion is one of the structures contributing to the excited state. 8-Aminofluoranthene shows the same phenomenon in its fluorescence spectra. In acidic dimethylformamide it absorbs as the cation at 362 nm and emits as the neutral amine at 515 nm. In the 10-nsec duration of excitation, which is long enough for several thousand collisions, the deprotonation has taken place.

Some of the types of compounds that are stronger bases in the excited state are carboxylic acids, aromatic ketones, aromatic aldehydes, and aza heterocyclic compounds. These compounds show an increase in the electron density of the O or N atom in the excited state, as shown for benzoic acid.[19]



This increased basicity in the excited singlet state has been reported for acetophenone[20] and 9-acridanone.[21] Both are air pollutants. In the determination of 9-acridanone in air, a methanol–trifluoroacetic acid (4:1) solution of the compound showed the excitation spectrum of the neutral compound and the emission spectrum of the cation, Fig. 4.[21] On the other hand, in strongly alkaline solution the excitation and emission spectra are derived from the anion.

This phenomenon has been used to characterize and determine atmospheric 7*H*-benz[*de*]anthracen-7-one.[22] An example of this type of analysis is shown in Fig. 5. In this case a two-dimensional alumina thin-layer chromatographic (TLC) separation of organic airborne particulates was followed by direct fluorimetric analysis after treatment with trifluoroacetic acid fumes. The excitation spectra were those of the neutral compound, and the emission spectra were those of the cationic salt.

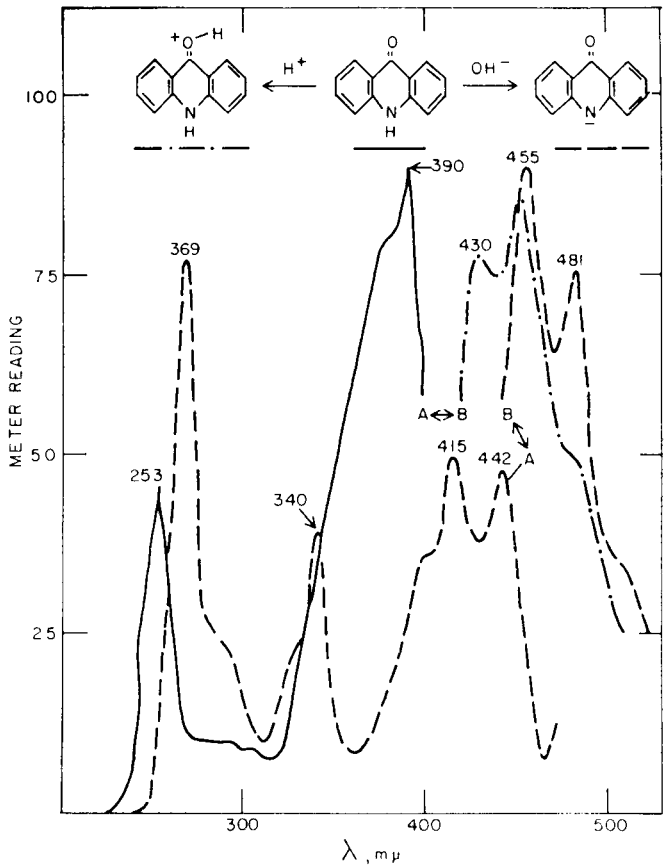


FIG. 4.—Spectrofluorimetric excitation (A) and emission (B) spectra of 0·2 μg of 9-acridanone/ml of solution at meter multiplier setting (MM) 0·01.
(– – – –) In dimethylformamide containing 5% of 10% aqueous tetraethylammonium hydroxide, F 269/481. Excitation (———) and emission (— · — ·) spectra of 9-acridanone in methanol-trifluoroacetic acid (4:1) at F 390/450.

2-Hydroxybiphenyl and 4-hydroxybiphenyl have been found in coal tar pitch.[23] These compounds can be readily differentiated and characterized. *o*-Hydroxybiphenyl shows excited state ionization, pK_a 10·0 and pK_a* 1·5, but the *p*-isomer does not show this phenomenon and has pK_a 9·5. At pH 12 both fluoresce as anions, but at pH 6 *p*-hydroxybiphenyl fluoresces in the molecular form, λ_{max} 340 nm, whereas the *o*-isomer fluoresces as its anion at 415 nm.[24]

Spectra of anions. Although compounds such as the carbazoles and 9-acridanone

are weak acids, they ionize as anions more readily in the excited state. This phenomenon has been used to characterize and estimate the amounts of carbazole, 11*H*-benzo[*a*]-carbazole, and 7*H*-benzo[*c*]carbazole in air polluted by coal-tar pitch fumes.[25] Atmospheric 9-acridanone,[21] unlike the analogous xanthan-9-one,[26] also has been characterized with the help of the fluorescence excitation and emission spectra of its anion.

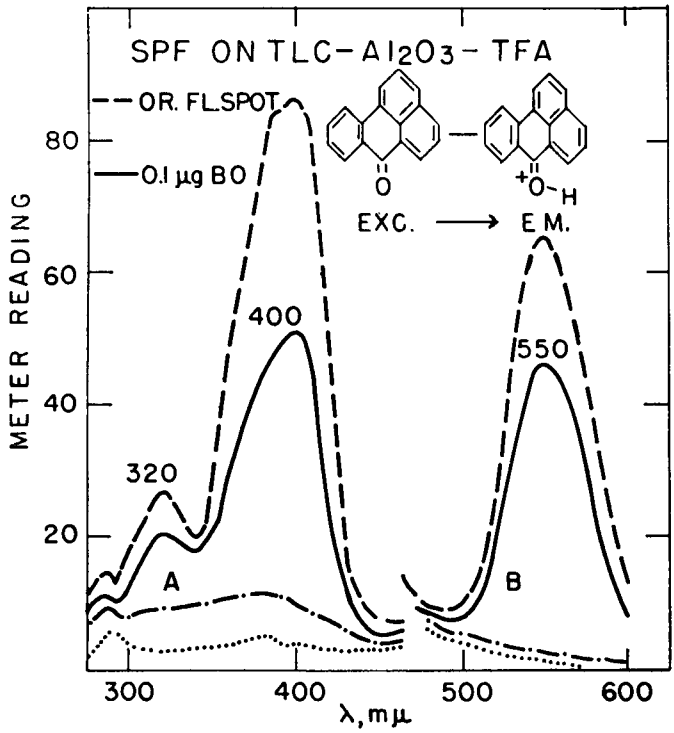


FIG. 5.—Fluorescence excitation (A) and emission (B) spectra at MM = 0·1, on the chromatogram of 0·1 µg of 7*H*-benz[*de*]anthracen-7-one (———); and an orange fluorescent "unknown" spot (– – – –). Standards and unknown treated with 0·02 ml of trifluoracetic acid.[22] (Reprinted by permission of the copyright holders, from *Mikrochim. Acta*, 1965, 1110.)

The fluorescence excitation and emission spectra of carbazole and its anion, Fig. 6,[27] and 9-acridanone and its cation and anion, (Fig. 4) have proved invaluable in these characterizations. 2-Fluorenol has been isolated from urban airborne particulates after aqueous alkaline extraction and several TLC separations and then characterized by fluorimetric examination of the neutral and alkaline solutions. (Fig. 7).[23]

Many polynuclear phenols have been found to be fluorescent in neutral and alkaline solution,[28] as have a variety of heterocyclic phenols.[23] With the help of TLC R_f values, paper electrophoretic mobility values, and fluorescence spectra in neutral and alkaline solution, the following phenols were identified in coal-tar pitch: 1-naphthol, 2-naphthol, 2-hydroxybiphenyl, 4-hydroxybiphenyl, 2-fluorenol, 3-fluorenol, 2-hydroxydibenzofuran, and 2-hydroxycarbazole.[23] 2-Fluorenol was

identified similarly in a New York airborne particulate sample. Scopoletin or 7-hydroxy-6-methoxycoumarin has also been identified in atmospheric and air pollution samples partially on the basis of the fluorescence spectra of its anion.[29]

Another class of compounds which are intensely fluorescent as anions comprises the polynuclear aromatic amines, many of which are carcinogenic.[15] A comparison of the fluorescence spectra of neutral 8-aminofluoranthene and of its cation and

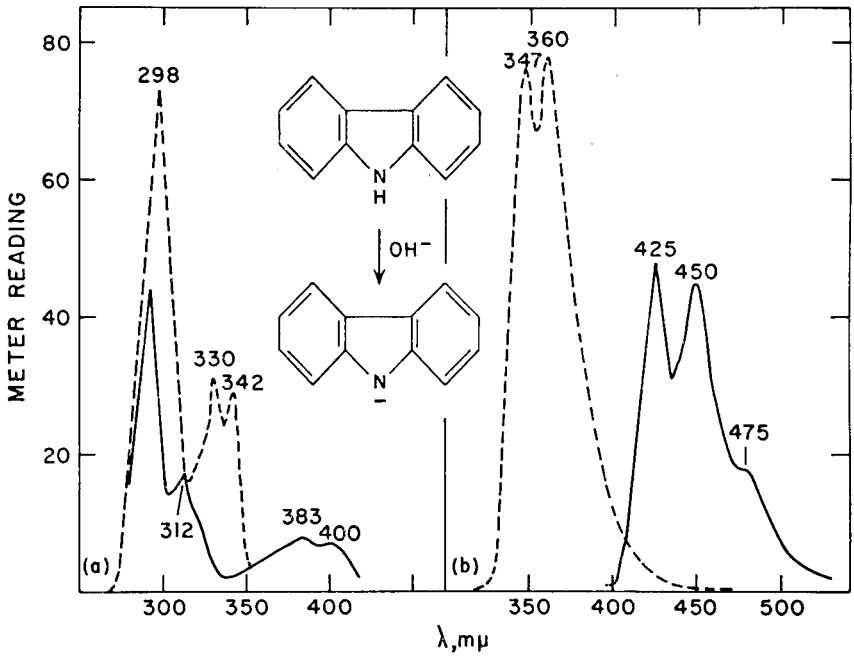


FIG. 6.—Fluorescence excitation (a) and emission spectra (b) of carbazole, $5 \times 10^{-7}M$ in dimethylformamide (- - - -) at MM = 0·01 and $10^{-6}M$ in alkaline dimethylformamide (———) at MM = 0·03.[27]

anion indicates that the anion (F 340, 412, 555/590) absorbs and emits at much longer wavelengths. The brilliant fluorescence of some of the aromatic acylamine anions should prove useful in the identification and determination of aromatic amines and their acyl derivatives.

For future application to air pollution analysis some new methods have been developed for the fluorescence analysis of various aliphatic aldehydes and their precursors.[30] Dimedone and 1,3-cyclohexanedione were used as reagents, with two procedures for each, one resulting in a neutral, and the other in an anionic fluorogen. The determination limit is about 30 ng of formaldehyde.

Malonaldehyde can be determined spectrophotofluorimetrically as an anionic fluorogen by reaction with electronegatively substituted aniline derivatives.[31] With the help of an oxidative step, the method has been applied to the characterization of deoxy sugars, such as 2-deoxyribose, 2-deoxygalactose, and 2-deoxyglucose, eluted from a chromatogram or a pherogram.[32]

Spectra of cations. Since cationic resonance structures absorb and fluoresce at longer wavelengths and usually with greater intensity than do comparable neutral

structures, many methods of analysis have been based on the formation of a fluorescent cation.

The benzopyrene fraction containing benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*k*]-fluoranthene and perylene is one of the fractions obtained in alumina column chromatography of organic airborne particulates.[33] When a sulphuric acid solution of this fraction is excited at 362 nm, the emission spectrum of a benzo[*e*]pyrene cation,

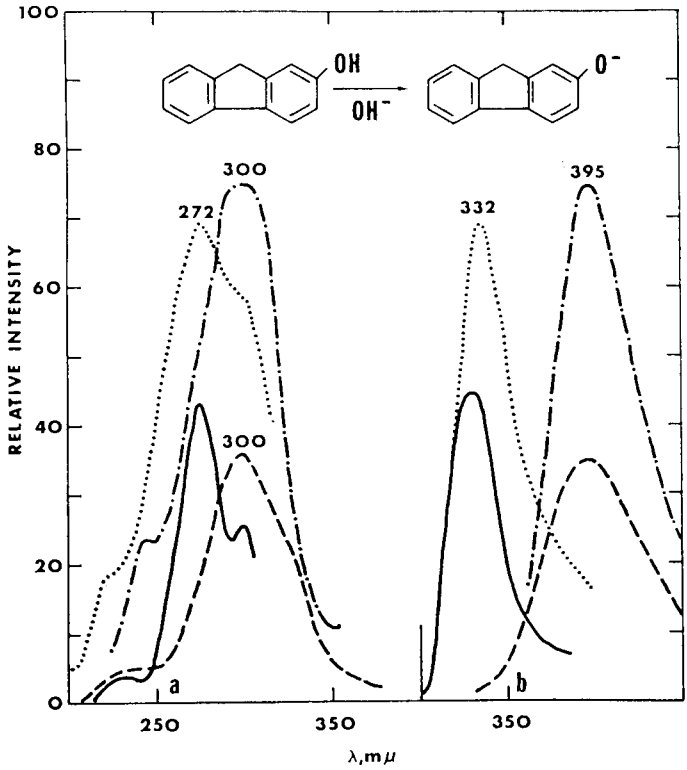


FIG. 7.—Fluorescence excitation (a) and emission (b) spectra at MM = 0·01, of 2-hydroxyfluorene and its anion.
(———) $2 \times 10^{-6}M$ in methanol at F 272/332. (– – – –) $2 \times 10^{6}M$ in methanol containing 3% of 40% aqueous tetraethylammonium hydroxide at F 300/395. Spot obtained after 4-TLC separations, elution with acetone and evaporation. (. . . .) Diluted with methanol to give MM = 0·01 at F 272/332. (— · — ·) Drop of 40% aqueous tetraethylammonium hydroxide added to about 1 ml of the methanolic solution and read at F 300/395.[23]

λ_{max} 391, 410, 427s, is obtained. In this fashion benzo[*e*]pyrene is characterized. The very distinctive fluorescence excitation and emission spectra of the benzo[*a*]-pyrene cation are obtained from the sulphuric acid solution either with the emission monochromator set at 540 nm or the excitation monochromator set at 470 nm (Fig. 8).[34] Less than milligram amounts of organic airborne particulates can be analysed for benzo[*a*]pyrene through this fluorescence reaction, following liquid–liquid extraction or TLC.

In Table II a larger group of methods for the determination of benzo[*a*]pyrene

is compared. The most sensitive, selective, and rapid methods are those involving the fluorescence of the benzo[*a*]pyrene cation.

A large number of polynuclear aza heterocyclic compounds have been found and determined in urban atmospheres[35] and in air pollution source effluents.[36–38] These compounds were characterized by R_f values, absorption spectra, and fluorescence spectra of the neutral compound and its cation. Although the fluorescence

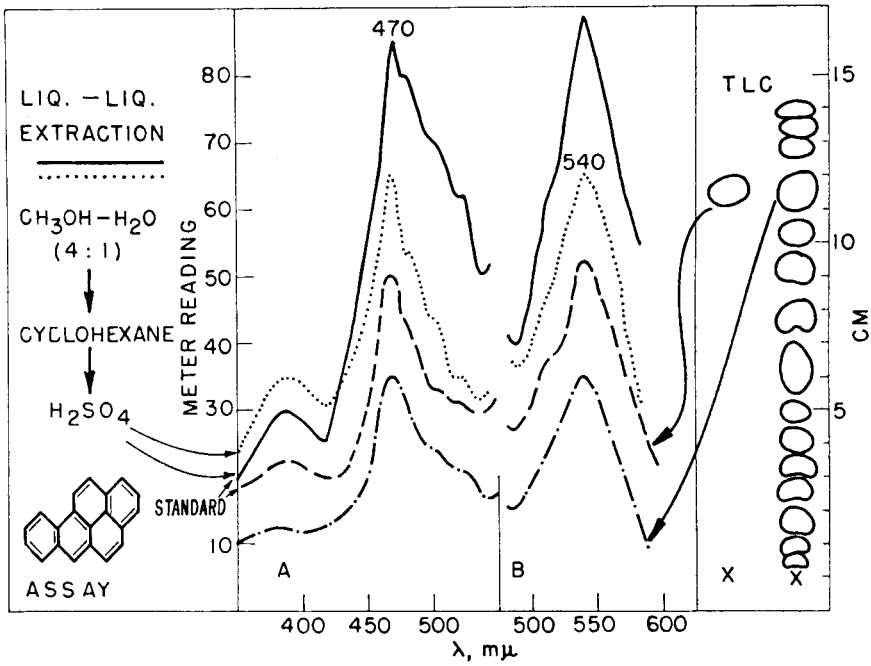


FIG. 8.—Comparison of the fluorescence excitation (A) and emission (B) spectra of various species in concentrated sulphuric acid.
On the left, liquid–liquid extraction procedure. On the right, alumina TLC separation of 100 μg of the benzene-soluble fraction and of pure BaP, pentane–ether (19:1) as developer. In the centre, the fluorescence spectra obtained at F 470/540. (— · — ·) Extract of the spot from the thin-layer chromatogram of the benzene-soluble fraction, MM = 0·003. (· · · ·) Fraction from the liquid–liquid extraction of 500 μg of benzene-soluble fraction, MM = 0·01 (———) BaP (0·25 μg) taken through the liquid–liquid extraction, MM = 0·01. (– – – –) Extract of the pure BaP (0·15 μg) spot from the thin-layer chromatogram, MM = 0·01.[34] (Reprinted by permission from *Atmos. Environ.*, 1967, **1**, 131.)

spectra of the neutral aza arenes have more fine structure, the cationic salts absorb and emit at longer wavelengths.[39] Atmospheric benz[*c*]acridine has been identified from similarity of the spectra of a standard and the sample cationic salt.[40]

Nitro- and amino-arenes can be readily characterized on glass-fibre paper or in solution through comparison of the fluorescence spectra of the derived iso-pi-electronic amine salt and of the parent hydrocarbon,[41] *e.g.*, the reduction product of 1-nitropyrene, 1-aminopyrene, and pyrene give very similar excitation and emission spectra in acid solution.

Carbonyl compounds can be characterized and determined fluorimetrically by a variety of methods. Thus, 1,4-cyclohexanedione can be determined by condensation with *o*-phthalaldehyde.[42] The fluorogen pentacenequinone was determined as the

TABLE II.—COMPARISON OF METHODS FOR THE DETERMINATION OF BENZO[*a*]PYRENE IN A COMPOSITE URBAN AIRBORNE PARTICULATE SAMPLE

Method	Detn. limit,* μg BaP	Benzene-soluble fraction, *mg*	Anal. time *hr*†		Concentration benzene soln., $\mu g/g$
			Sepn.	Anal.	
1. CC sepn.: SP‡	10	100	3§	14	850; 920
2. TLC on Al_2O_3/cellulose acetate: SPF	0·003	0·05	3	0·5	950 ± 200¶
3. Two-way TLC on Al_2O_3/cellulose acetate: SPF	0·003	0·05	4	0·5	800 ± 300
4. TLC on Al_2O_3: SPF in H_2SO_4	0·003	0·05	1	0·5	800 ± 50
5. TLC on Al_2O_3/cellulose acetate: SPF in H_2SO_4	0·003	0·05	3	0·5	850 ± 50
6. CC: SPF in H_2SO_4	0·5	5	2	0·2	1000 ± 100
7. H_2O/CH_3OH(1:4): cyclohexane: H_2SO_4: SPF	0·12	0·5	1	0·1	900 ± 200
8. TLC on Al_2O_3: F in H_2SO_4	0·01	0·05	1	0·2	950 ± 100
9. TLC on Al_2O_3/cellulose acetate: F in H_2SO_4	0·01	0·05	3	0·2	800 ± 150
10. Hexane: H_2SO_4: SPF	0·01		1	0·1	900 ± 400
11. TLC: GLC	5	15	>2	>1	690; 750

* Determination limits vary with concentration of BaP in the sample. Thus, with pure BaP the limit would be about 2 μg for method 1.
† All analysis times are for individual analysis and are approximate. Analysis includes spectral analysis and calculations.
‡ Method for 8–12 aromatic hydrocarbons: CC = column chromatography; SP = spectrophotometry; SPF = spectrophotofluorimetry; F = fluorimetry.
§ Plus evaporation overnight.
¶ Eight determinations for this and subsequent methods. The range is given.

dicationic salt in concentrated sulphuric acid solution. The main excitation bands are at 350, 575 and 625 nm, a single emission band is found at 650 nm.

Phenalen-1-one (PO) and the carcinogen 7*H*-benz[*de*]anthracen-7-one (BO) have been characterized and assayed in air and in air pollution source samples by the use of the fluorescence spectra of the cations.[22,43] The methods used in the analysis for BO are listed in Table III. The nitro-derivative tends to reduce the background fluorescence present in the blank by quenching. In the filter fluorimetric method, a high concentration of a powerful quenching agent is necessary. Otherwise, the blank fluorescence is so high that the method cannot be used.

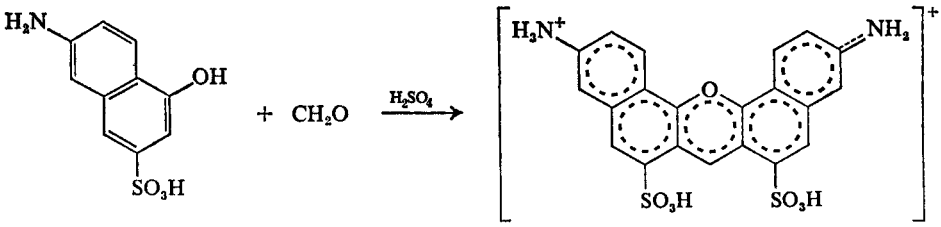
Atmospheric 9-acridanone can be determined fluorimetrically as the cation after two-dimensional TLC separation [21] (see Fig. 4 for spectra obtained). Since the excitation spectrum is that of the neutral compound and the emission spectrum is that of the cation, the analytical excitation and emission wavelengths are widely separated, so scatter is less of a problem and the analysis performed more readily.

Formaldehyde and acrolein can be determined with J-acid.[44] The fluorogen from the reaction of formaldehyde with J-acid is a blue dibenzoxanthylium dication which has a green-yellow fluorescence and is yellow in sulphuric acid.

TABLE III.—COMPARISON OF METHODS FOR THE DETERMINATION OF ATMOSPHERIC BENZANTHRONE[22,50,59]

Adsorbents	Developers	Test solution	Wavelength, *nm*		Total anal. time, *hr**
			Excit.	Emiss.	
Al_2O_3	CH_2Cl_2	2% 2-nitrothiophene in TFA†	495	555	2
Al_2O_3	CH_2Cl_2	3% *m*-dinitrobenzene in TFA	495	555	2
Al_2O_3	CH_2Cl_2	TFA	495	555	2
Al_2O_3	CH_2Cl_2	H_2SO_4	495	555	2
Al_2O_3	Cy + EtOAc(1:1), then toluene‡	3% *m*-dinitrobenzene in TFA	495	555	2·5
Al_2O_3	Cy + EtOAc(1:1), then toluene‡	TFA	495	555	2·5
Al_2O_3	Cy + EtOAc(1:1), then toluene‡	§	400	550	2·5
Al_2O_3¶	CH_2Cl_2	15% *m*-dinitrobenzene in TFA	Prim. filter peaks 405	Sec. filter peaks 546	2
GFP–SiGel**	Pentane–CH_2Cl_2(3:1)	H_2SO_4	360††	560	0·5
GFP–SiGel**	Pentane–CH_2Cl_2(3:1)	‡‡	Estimation by eye		0·3

* Starting from the organic particulate fraction.
† TFA = trifluoroacetic acid.
‡ Two-dimensional TLC; Cy = cyclohexane.
§ Direct SPF.
¶ Filter fluorimetric method.
** Instant TLC; GFP = glass fiber paper.
†† With filter that excludes wavelengths below 535 nm.
‡‡ Direct examination of chromatogram under long-wavelength ultraviolet light.



F470/520

In the determination of acrolein the fluorescence intensity was stable for at least 90 min. The standard deviation was 1·4% (21 variates). The calibration curve was linear from 0·25 to 7 μg of acrolein and from 0·01 to 2 μg of formaldehyde.

The J-acid method has been used to determine polyols and sugars, after periodic acid oxidation, Table IV.[45] The method is especially sensitive for formaldehyde precursors, such as the polyols and 2-deoxyribose.

In a comparison of the J-acid procedure and five others the 2,4-pentanedione method was found to be the most highly selective for formaldehyde.[30] The J-acid method was less selective but more sensitive.

The J-acid method has also been used for the oxidative assay of formaldehyde precursors, such as polyols, sugar acids, 2-deoxy sugars, ketoses, 1-alkenes, 1-alkenols, acrylic acid, and corticosterones.[46]

TABLE IV.—FLUORIMETRIC DETERMINATION AT F 463/522 OF FORMALDEHYDE PRECURSORS* BY THE J-ACID METHOD[45]

Compound	MM · T	Compound	MM · T
Ethylene glycol	1·53	2-Deoxyribose	0·50
Propylene glycol	1·03	Rhamnose	0·18
Glycerol	0·85	Ribose	0·06
Dulcitol	0·50	Adenosine	0·033

* Final concentrations of all compounds $5 \times 10^{-6} M$.

Complex formation

Although excited state dimers, or excimers, can be formed in solution, the solution has to be so concentrated that the fluorescence spectrum of the excimer can be used only with difficulty in trace analytical work. However, minute amounts of many chemicals can give fluorescence spectra of their dimers on chromatograms and pherograms. The fluorescence spectra of excimers are therefore discussed in the section on solid state fluorescence.

Another type of fluorescence found in some complexes obtained with polynuclear compounds is charge-transfer fluorescence.[47] Essentially, a complex is formed with an electron-acceptor, such as benzo[*a*]pyrene, and an electron-donor, such as dimethylaniline. Since the complex is stable only in the excited state, the excitation spectrum is that of the polynuclear compound and the emission spectrum contains bands from the polynuclear compound, and a longer-wavelength featureless band derived from the excimer. The relative proportions of these two sets of bands depend on the solvent and on the structures and concentrations of the polynuclear compound and of the complexing agent.

An example is the charge-transfer complex formed between benzo[*a*]pyrene and dimethylaniline in methylene chloride. Increasing amounts of dimethylaniline decrease the quantum yield of fluorescence of benzo[*a*]pyrene, Fig. 9. With high concentrations of dimethylaniline, the fluorescence emission of benzo[*a*]pyrene is quenched but that of the complex is relatively unaffected. Other polynuclear compounds which form fluorescent charge-transfer complexes are anthracene, pyrene, fluoranthene, benzo[*e*]pyrene and perylene. Since the spectra of complexes can be obtained at very low concentrations of polynuclear compound, the phenomenon should prove useful in trace analysis.

Photodecomposition

Since ultraviolet light, especially at short wavelengths, is of high enough energy to break bonds, photodecomposition and photosynthesis during moderately prolonged fluorimetric examination are not unusual phenomena. Most fluorescence assays are carried out on dilute solutions containing from 0·1 ng to 10 μg of test substance, so especially if the fluorimetric examination is prolonged, ample light energy per molecule per unit time is available to allow the test substance to undergo photodecomposition or to react with itself or other molecules in the solution.

An increase in the intensity of a light source enhances the sensitivity of an instrument but can also increase the extent of photodecomposition. However, reasonably rapid measurements can overcome these difficulties. A change in solvent can also change the rate of decomposition.

Many examples of photodecomposition are known. A standard solution of

quinine bisulphate (10 ng/ml) is unstable in ultraviolet light. Although 3-hydroxypyridine is stable to light, its derivatives pyridoxine and pyridoxal and its 5′-phosphate, in amounts of 2 μg/ml, at pH 6·8 undergo a decrease in fluorescence intensity on 5 min or more of exposure to ultraviolet light.[24] The paper electrophoresis, in a well-lighted room, of scopoletin with 0·1*M* sodium hydroxide as electrolyte, results in eventual destruction of the scopoletin.

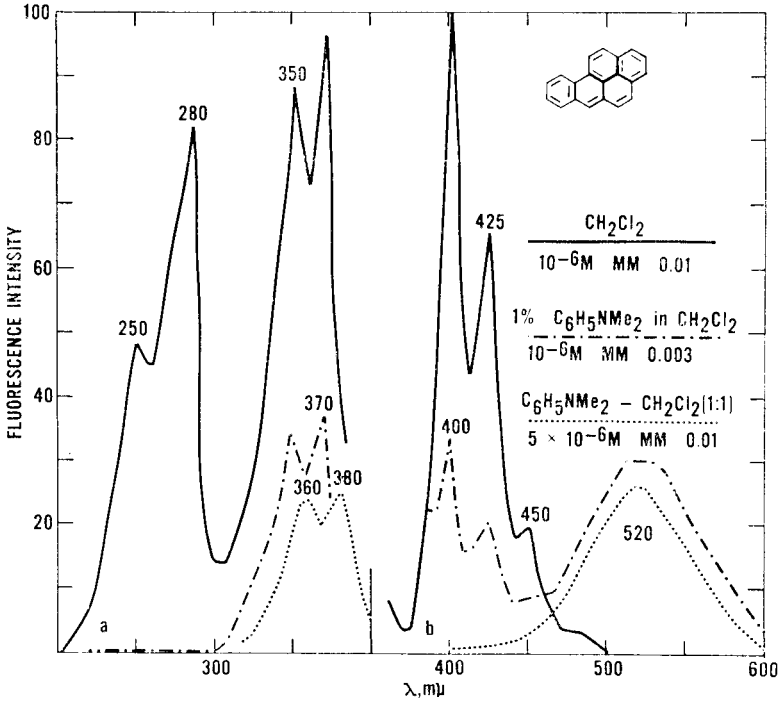
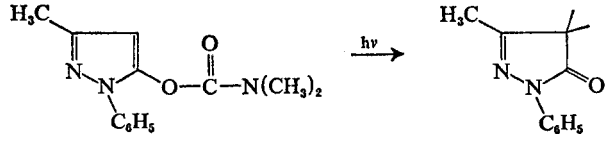


FIG. 9.—Fluorescence excitation and emission spectra of benzo[*a*]pyrene, $10^{-6}M$ (———) in methylene chloride at MM = 0·01 and F 350/400; (– – – –) in methylene chloride–dimethylaniline (99:1 v/v) at MM = 0·003 and F 370/520; (· · · ·) in methylene chloride–dimethylaniline (1:1) at $5 \times 10^{-6}M$ BaP, MM = 0·01 and F 380/520.

The presence of insecticides in the atmosphere has never been adequately investigated, although it can be assumed that in parts of the country where spraying takes place, fairly large concentrations are probably present. The photodecomposition of these compounds is thus of interest. The behaviour of five carbamate insecticides under irradiation with natural sunlight and with short-wavelength ultraviolet light has been studied.[48] Pyrolan (1-phenyl-3-methyl-5-pyrazolyldimethylcarbamate) in alcohol gave 1-phenyl-3-methyl-5-pyrazolone after 5 min irradiation. Increases in the irradiation time gave three additional decomposition products. Pyrolan was more stable to sunlight irradiation.



A cyclohexane solution of 1-naphthol at a concentration of 10 μg/ml has a half-life of only 1·5 min when decomposed by ultraviolet irradiation; in contrast, a solution of carbaryl (1-naphthyl-*N*-methylcarbamate) of the same concentration has a half-life of 7 min. TLC studies indicate that at least 4 products are formed, one of which is 1-naphthol. Photodecomposition of carbamates is delayed more in alcoholic solution than in cyclohexane solution.

Probably the most sensitive method for the determination of atmospheric benzo[*a*]-pyrene involves fluorimetric determination of a solution of the compound in sulphuric

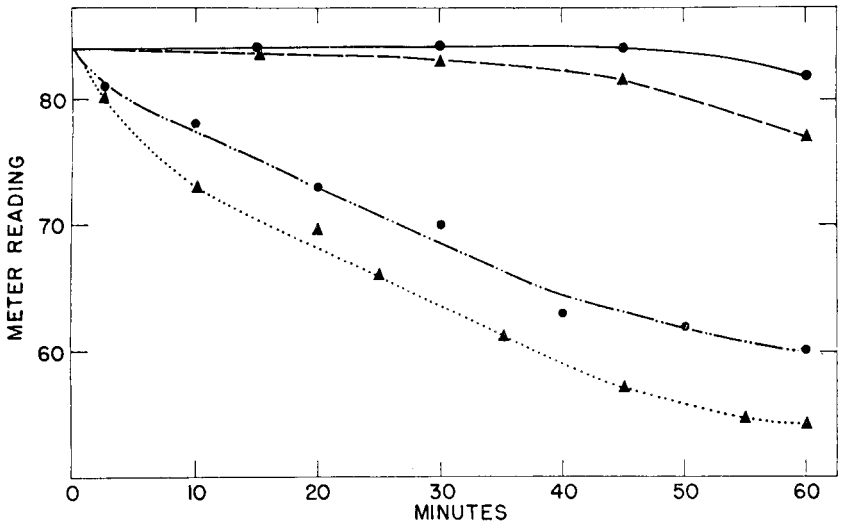


FIG. 10.—Effect of time on the intensity of the fluorescence wavelength maximum at 545 nm of a 2 × $10^{-6}M$ solution of benzo[*a*]pyrene in concentrated sulphuric acid. (———) Unilluminated solutions, activation at 520 nm; (— · · · —) steady illumination at 520 nm; (– – – –) unilluminated solutions, activation at 470 nm; (· · · ·) steady illumination at 470 nm.[2] (Reprinted by permission from *Intern J. Air Pollution*, 1960, **2**, 253.)

acid.[34] Consequently, the effect of light on the fluorescence intensity is of some importance (Fig. 10).[2] The decrease in fluorescence depends on the time of exposure and on the wavelength of the irradiation.

Photodecomposition can lead to the enhancement or production of fluorescence. Thus, an alkaline solution of coumarin is not normally fluorescent, but treatment with ultraviolet light results in the production of the fluorescent dianion of trans-*o*-hydroxycinnamic acid.[49] This effect is said to occur with other coumarin derivatives which lack a free OH group in the benzene ring.

SOLID STATE FLUORESCENCE

Recent years have seen the accelerated development of direct fluorimetric examination of paper and thin-layer chromatograms and pherograms. The ease and facility of examination result in the saving of much time and effort. Since only nanogram or microgram amounts of test substance are being examined, the effects of short range intermolecular phenomena, temperature changes, atmospheric oxygen, and light on the spectra assume major importance.

Direct spectrophotofluorimetry

Numerous authors have reported fluorescence measurements of spots on paper chromatograms.[51–54] Emission spectra of nine polynuclear aromatic hydrocarbons have been obtained on paper chromatograms after column and paper chromatography of urban organic airborne particulates.[55] The limit of detection was 1 μg. Fluorescence excitation and emission spectra of a variety of compounds on thin-layer chromatograms have been obtained,[56] and ng–μg amounts of material analysed. Elution or extraction of a spot is not necessary. Since many organic compounds can be separated from organic airborne particulates by TLC and then determined by direct fluorimetry, smaller volumes of polluted air can be analysed, much more quickly.

Usually, fluorescence spectra of a compound on a chromatogram or pherogram are closely similar to the analogous spectra of the compound in solution. However, there are some exceptions. Thus, benz[*c*]acridine on a cellulose plate has a broad fluorescence emission band at 405 nm and has bands at 384, 408, and 430 nm in pentane solution. For μg-amounts of some compounds in the solid state, on the chromatogram an excimer band is found in the emission spectra. In addition, energy transfer in the form of sensitized fluorescence can take place more readily on the chromatogram than in solution.

Their carcinogenic potential leads to interest in the analysis of many of the air pollutants, *e.g.*, arenes, aza arenes, imino arenes, aromatic amines, and ring-carbonyl compounds.

Aromatic hydrocarbons or arenes. Compounds coming off a gas chromatographic column can be collected on a thin-layer plate and then examined fluorimetrically. In this fashion polynuclear aromatic hydrocarbons isolated from polluted air can be readily characterized, as shown in Fig. 11 for the pyrene fraction obtained by preliminary column chromatographic separation.[5]

Aaz arenes. Many of these compounds have been found and determined in urban airborne particulates and in air pollution source effluents.[35,37] They have been characterized fluorimetrically on the thin-layer plate.[35,38,56]

Atmospheric benzo[*h*]quinoline and benz[*c*]acridine have been assayed by direct fluorimetric examination of two-dimensional, thin-layer chromatograms.[57] Atmospheric acridine can also be determined by direct fluorimetric examination of paper pherograms of organic airborne particulates.[58]

Aromatic amines. The fluorescence spectra of these amines can be obtained at nanogram concentrations from paper or thin-layer chromatograms and pherograms.[15]

Ring-carbonyl compounds. 7*H*-Benz[*de*]anthracen-7-one, a carcinogen present in urban atmospheres, has been isolated from the atmosphere and characterized on a thin-layer plate (Fig. 5). Following TLC, it has been assayed by fluorimetry directly on the plate or after elution.[22,43]

Atmospheric phenalen-1-one has been similarly characterized and assayed. However, a recent instant TLC procedure indicates that atmospheric phenalen-1-one and 7*H*-benz[*de*]anthracen-7-one can be assayed in minutes even with an elution step.[59] Atmospheric 9-acridanone is another carbonyl compound which can be assayed fluorimetrically on the plate or after elution.[21]

Aldehyde precursors. Urban airborne particulates have been shown to contain large quantities of furfural[60] and aldehyde[45,46] precursors. Apparently, these are

mainly α-glycollic-type compounds. In further development of this work, the location and fluorimetric analysis of 2-deoxy sugars on chromatograms and pherograms was studied.[32] The sugar was oxidized to malonaldehyde on the paper and then was combined with an aromatic amine or with 2-thiobarbituric acid. With 4′-amino-acetophenone, spectra of the neutral and anionic species are obtained; with 2-thiobarbituric acid, long-wavelength excitation and emission spectra are obtained.

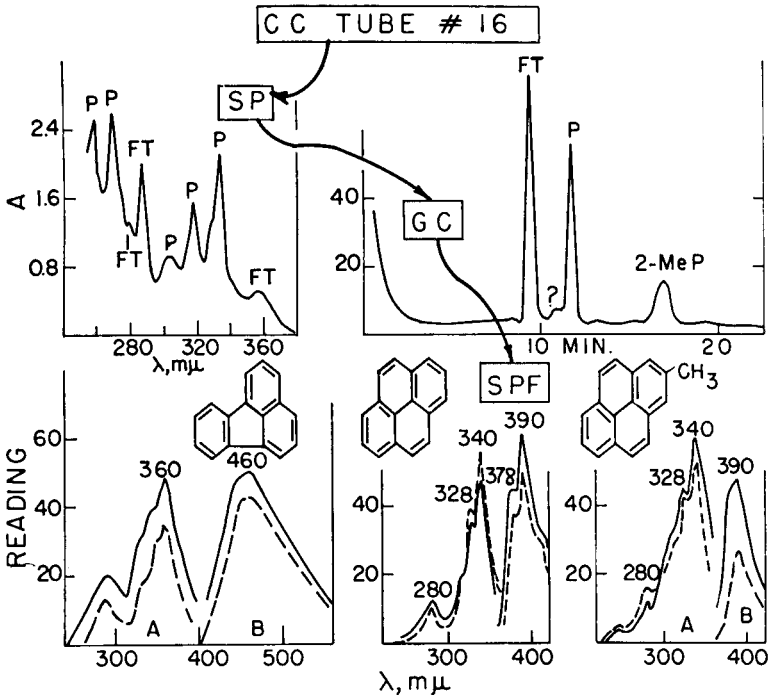


FIG. 11.—Alumina column chromatographic fraction 16 of benzene-soluble fraction of urban airborne particulates. Gas-liquid chromatogram. Fluorimetric spectra obtained from the spectral examination of the appropriate gas chromatographic eluates.[5]

Compounds that are oxidized or hydrolysed to formaldehyde by hot sulphuric acid can be determined on a glass-fibre paper chromatogram by the J-acid method.[61] Compounds such as *sym*-trithiane, hexamethylenetetramine, glyoxylic acid, piperonylic acid, 2,4,5-trichlorophenoxyacetic acid and monochloroacetic acid react.

Amino-acids and proteins. Preliminary investigations of the acid hydrolysis products of various types of airborne particulate samples have shown the presence of amino-acids. For direct fluorimetric investigation, dansylamino acids have been used.[62,63]

Wet and dry fluorescence

Many compounds differ in their fluorescence properties under ultraviolet light, depending on whether they are present on the chromatogram as a wet spot or a dry spot.[64] In the dry state, excimer formation, sensitized fluorescence, or excited state ionization can drastically change the fluorescence spectra of a compound. Examples are given later. For many compounds, quenching can take place when

the compound is in the solid state on the chromatogram; for other compounds quenching occurs when the compound is in solution on the chromatogram. For example, fluorescence spectra can be obtained from the dry spot of 9-acridanone, but not from a dry spot containing 1 μg of 7*H*-dibenzo[*c*, *h*]xanthen-7-one.[65] Fluorescence spectra can be obtained for phenalen-1-one in the dry state on the cellulose plate, but not when the plate is wet with methanol or with dimethylformamide.

Scanning

The direct fluorimetric scanning of paper chromatograms for quantitative estimations of various compounds has been reviewed,[66] and direct fluorescence scanning of thin-layer chromatograms has been used in quantitative investigations.[67,68] However, with the use of proper excitation and emission spectral bands, a chromatogram can be scanned for an individual compound or for a family of compounds with much greater selectivity and sensitivity than has hitherto been possible.[69]

This selective fluorescence scanning technique has been used in the determination of benzo[*a*]pyrene,[34] aza arenes,[69] phenalen-1-one, and 7*H*-benz[*de*]anthracen-7-one[22] after TLC. Fluorescence scanning has also been used to assay for atmospheric acridine after paper electrophoretic separation of organic airborne particulate samples.[58]

Excited state complexes

Various types of complexes can be formed which are stable in the excited state but not in the ground state. The complex can be a polymer and can have fluorescence characteristics entirely different from those of the test substance; *e.g.*, it could fluoresce at longer wavelength, or it could be non-fluorescent. This section discusses dimers that fluoresce at longer wavelength than do the monomers and, since they do not exist in the ground state, have only one broad, structureless emission band.

Excimers. Excimer formation was first noted by Forster and Kasper in 1955.[70] At concentrations of pyrene $>10^{-4}M$, a new broad fluorescence band appears at longer wavelengths and at concentrations of $\sim 10^{-2}M$ is more prominent than the monomer emission bands. The species responsible was postulated as a dimer, formed by combination of an electronically excited pyrene molecule with a pyrene molecule in its ground state. Consequently, it was named an excited state dimer or excimer. Many aromatic hydrocarbons and their derivatives are known to exhibit this behaviour. Excimer fluorescence has been discussed,[71] and an extensive compilation made of compounds which exhibit excimer fluorescence.[72]

Excimers are of great importance in direct fluorimetric analysis of chromatograms or pherograms. The material in a separated spot is usually at high concentration in the sense that the molecules are very close together because they are either in the solid state or in a highly concentrated solution. In the solid state, excimer formation may be due to the π-orbital overlap of adjacent molecules;[73] in concentrated solution, the formation of excimers is believed to be a diffusion-controlled process involving very effective collisions between excited and unexcited monomers.[74]

Excimer formation on thin-layer chromatograms has been described.[56] The fluorescence spectra of pyrene (0·1 μg) and of benzo[*a*]pyrene (0·5 μg) on a cellulose thin-layer plate showed excimer bands at 470 nm.

In the characterization of aromatic carbonyl compounds on a chromatogram by

reduction to an alcohol iso-pi-electronic to the parent hydrocarbon, excimer formation can take place. This has been shown for less than μg-amounts of 1-pyrene aldehyde and 1-acetylpyrene.[41]

Charge-transfer fluorescence. Even on a chromatogram, arenes can form charge-transfer complexes with dimethylaniline, as shown for anthracene in Fig. 12. The excitation spectrum is derived from the anthracene monomer, and the emission spectrum is derived from the anthracene–dimethylaniline excited state complex.

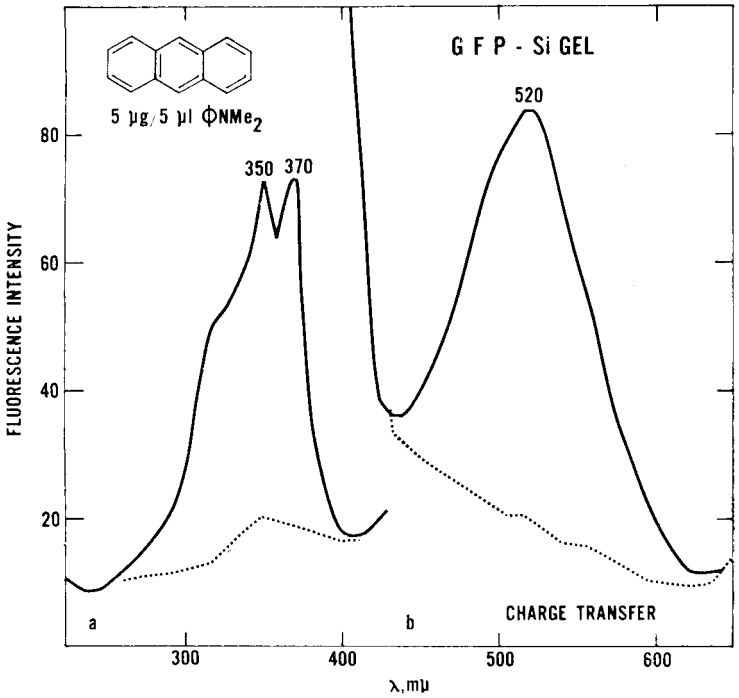


FIG. 12.—Fluorescence excitation and emission spectra of anthracene (5 μg) in dimethylaniline on glass-fibre paper impregnated with silica gel at MM = 0·001.

This complex is stable only in the excited state. The analytical usefulness of these complexes has not been fully explored.

Sensitized fluorescence

Under appropriate conditions, singlet–singlet energy transfer of absorbed radiation can result in fluorescence quenching or in sensitized fluorescence. The various processes have been discussed.[71] Essentially, the major component of a mixture absorbs light and then transfers it to the minor component which then emits with increased intensity at much longer wavelengths. For maximum efficiency, the fluorescence spectrum of the major component overlaps the absorption spectrum of the minor component. This phenomenon has been used to identify traces of naphthacene in anthracene.[75]

At present, traces of impurities in standards hamper research investigations by the air pollution analyst. Some of the reported fluorescence spectra of supposedly pure arenes on paper and thin-layer chromatograms show emission bands at longer

wavelengths than those derived from the pure arene. Thus, high concentrations of benz[*a*]anthracene and dibenz[*a*,*h*]anthracene and very high concentrations of coronene on paper show long-wavelength emission spectra attributed to self-quenching or autoabsorption.[55] Actually, the long-wavelength bands from the anthracene and benz[*a*]anthracene spots are the emission spectra of tetracene and are examples of sensitized fluorescence. The same phenomenon has been shown for anthracene and benz[*a*]anthracene in 0·2 and 0·5 μg amounts on a cellulose thin-layer plate.[56]

It must be emphasized that it is only trace amounts of impurity that can cause difficulty. For example, "pure" benz[*a*]anthracene contains only trace amounts of tetracene, yet in a spot on a cellulose thin-layer plate containing 0·5 μg of benz[*a*]-anthracene and only 60 pg of tetracene, the emission bands of tetracene at 482, 520, and 570 nm are obvious and definite, whereas pure tetracene on a similar plate is at most weakly fluorescent. This difference in fluorescence between pure tetracene and a high dilution of it in another species explains why this phenomenon is called sensitized fluorescence.

Photodecomposition

Prolonged exposure of chromatograms and pherograms to ultraviolet light or even to room lighting can result in photodecomposition of some compounds. Of 141 pesticides (10 μg on paper) exposed to germicidal light (30 min on each side), 30 showed little change, 32 were completely degraded, and the remainder yielded either unsatisfactory spots or no spots.[76] Observations have been made on spots of 15 representative hydrocarbons on various thin-layer adsorbents following exposure to ultraviolet light.[77] On silica gel G and on aluminium oxide G, spots of hydrocarbons with an anthracenic structure turn yellow or a yellow-tan; spots of peri-condensed hydrocarbons (*e.g.*, benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, *etc*) turn darker tan or brown. The presence of a chlorinated solvent accelerates the decomposition. Among the numerous products of the exposure to ultraviolet light of a spot of pyrene on silica gel G were 1,6-pyrenedione and 1,8-pyrenedione, indicating that photo-oxidation must take place. Hydrocarbons, such as phenanthrene, chrysene, tri-phenylene, and picene, showed only a slight fading in fluorescence after standing under room lighting for several days. 7*H*-Benz[*de*]anthracen-7-one on an alumina plate exposed to room lighting for 20 hr or to ultraviolet light for 15 min showed no decomposition.[50]

Caffeine-impregnated silica gel plates can readily decelerate photo-oxidation of the arenes.[78] In one reported experiment a caffeine-impregnated silica gel plate, an untreated alumina plate, and an untreated silica gel plate were each spotted with 6 arenes and then developed with light petroleum–pyridine, exposed to air and daylight, and repeatedly examined in ultraviolet light (360 nm).[79] On the untreated plates after 1–2 hr the spots darkened and the fluorescence weakened (anthracene and pyrene) or almost disappeared (benzo[*a*]pyrene, benzo[*ghi*]perylene, chrysene and perylene), but on the caffeine-impregnated plate they were completely unchanged after 4 days.

Of course, if precautions are taken to expose the plate to a minimum of light for minimum time, analyses can be performed readily with good to excellent recoveries.

Imino heterocyclic compounds treated on a thin-layer plate with trifluoroacetic acid fumes under ultraviolet light are photo-oxidized to brilliantly coloured spots.[15]

Thus, carbazole gives a blue spot and phenothiozine a brown. This photo-oxidative procedure has been used in the characterization of atmospheric imino heterocyclic compounds and as a marker in investigating organic airborne particulates after one-dimensional[80] and two-dimensional[5] separation on alumina thin-layer plates.

ASSAY METHODS

In this section a few of the aspects of fluorescence analysis are briefly discussed. These include selectivity, sensitivity, speed and simplicity, precision, interference, relation between concentration and fluorescence intensity, and scanning.

Selectivity

The various photometric methods of analysis increase in selectivity in the approximate order—ultraviolet absorptiometry $<$ visible absorptiometry $<$ colorimetry $<$ spectrophotofluorimetry $<$ quenchofluorimetry $\sim$ spectrophotophotophosphorimetry $<$ quenchophosphorimetry. This order is based on the facts that more compounds absorb ultraviolet light than absorb visible light and that fewer compounds can be modified by a reagent to form a coloured derivative. Not all compounds that absorb light emit it in the form of fluorescence. Non-radiative processes can dissipate the energy or transfer it by intersystem crossover to the triplet state. Many of the compounds that fluoresce can be quenched selectively.[81] Before phosphorescence can take place, many more processes can interfere to dissipate the light energy. Consequently, since fewer compounds phosphoresce than fluoresce or absorb, phosphorescent methods are the most highly selective of the photometric methods, and since many families of phosphorescent compounds can be quenched selectively,[82] quenchophosphorimetry is the most highly selective of the photometric methods.

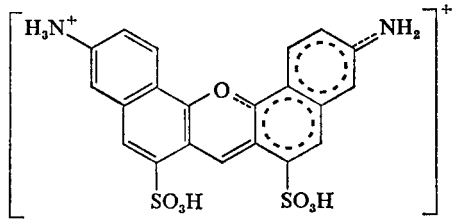
The selectivity of a fluorescent method of analysis can be improved by setting the two monochromators at wavelengths where there is a minimum of background fluorescence and a fairly high fluorescence intensity of the test substance. Thus, it is possible to obtain the pure fluorescence emission (or excitation) spectrum of a compound in a mixture by determining the spectrum of the mixture at an appropriate excitation (or emission) wavelength.

Selectivity in atmospheric analyses by fluorimetric assay can also be improved through preliminary separation by liquid–liquid extraction,[35–38] TLC,[21,22,25,34,83,84] paper electrophoresis,[23,58] column chromatography[2,33–38,65,66,85–97] and paper chromatography.[55,98,99]

Quenchofluorimetric techniques can also be used to improve selectivity in the determination of an air pollutant, *e.g.*, benz[*c*]acridine and benz[*h*]quinoline[15] and 7*H*-benz[*de*]anthracen-7-one.[43]

In the determination of the first member of a family of compounds, selectivity can be improved by picking a reagent which will react only with the first member because of that member's greater reactivity and smaller size. Thus, J-acid can be used to determine formaldehyde in the presence of other alkanals.[44] The reaction of the other aldehydes with the reagent is sterically hindered by the large sulphonic acid groups, as shown for the fluorogen. However, compounds decomposing to formaldehyde in warm sulphuric acid will also react,[100] as will acrolein.[44] In the case of acrolein, the addition of water to the final solution deprotonates the formaldehyde

fluorogen to give the non-fluorescent blue monocation, while the fluorescence of the acrolein fluorogen is unaffected.



Another reagent that can be used for the selective determination of formaldehyde, and would probably be ideal for atmospheric analysis for it, is 2,4-pentanedione.[101] Other β-diketones are much less selective since they react with the aliphatic aldehydes also.[30]

This selectivity has been used to determine formaldehyde-precursors such as α-glycollics, polyols, sugars, 1-alkenes, *etc.*, by oxidative J-acid[45] and 2,4-pentanedione[30] methods.

Sensitivity

In the determination of benzo[*a*]pyrene, the fluorimetric method is at least 100 times more sensitive than the absorptiometric method. Formulae have been established to calculate the absolute fluorescence sensitivity of a fluorescent molecule.[102] Sensitivity depends in part on instrumental factors such as stray light, intensity and spectral distribution of the light source, size and band-width setting of monochromators, characteristics of the photomultiplier and of the amplifier, and magnitude of background fluorescence from solvent, reagents, cuvette, adsorbent, *etc.*[102,103] The sensitivity and resolution of a spectrophotofluorimeter can be conveniently and rapidly checked by measurement of the Raman spectrum of a solvent.[107,104]

The sensitivity of a compound can be altered on ionization. Thus, the neutral monocyclic phenols fluoresce although their anions do not; simple anilines fluoresce although their cations and anions do not.[24] In the polynuclear compounds, the changes are not as drastic, but changes in band shape and intensity do occur. For example, 1-aminopyrene has about four times the fluorescence intensity of the cation,[80] and the 2-naphthol anion is about four times as fluorescent as the neutral molecule.

The fluorescence intensity or sensitivity of a fluorescent compound can be changed drastically by a change in solvent. In addition, a change in the structure of a molecule can change the fluorescence intensity (Table I).

Speed and simplicity

The complexity of the procedures for analysis of organic airborne particulates decreases in the order (column chromatography + spectrophotometry) > (column chromatography + gas chromatography) > (paper chromatography + elution + spectrophotofluorimetry) > (TLC + elution + spectrophotofluorimetry) > (TLC + direct spectrophotofluorimetry) > (instant TLC + elution + spectrophotofluorimetry) > (instant TLC + direct spectrophotofluorimetry) > colorimetry = fluorocolorimetry > direct absorptiometry $\sim$ direct fluorimetry. However, as the speed and simplicity of the methods improve, the selectivity worsens.

When it is possible to substitute instant TLC procedure (*e.g.*, glassfibre paper chromatography) for a TLC procedure, the separation time is reduced from 1–2 hr to 10–20 min. A comparison of the two types of separation in the analysis of atmospheric phenalen-1-one and 7*H*-benz[*de*]anthracen-7-one shows the convenience of the instant TLC methods. Eight methods for BO by TLC and various spectrophotofluorimetric (SPF), quenchofluorimetric and filter fluorimetric procedures average 2–2·5 hr per analysis.[43] However, with instant TLC + elution + SPF methods, a dozen organic airborne particulate samples can be analysed in the same time.[59] With measurement by eye or with direct fluorimetric scanning, the time can be shortened to about 20 min.

Precision and accuracy

The accuracies of most of the analytical methods for the analysis or organic airborne particulates have been checked by recovery experiments. Known amounts of a pure standard are added to a particulate sample, and the sample is analysed by the procedure under investigation. This has been done for air pollutants such as 9-acridanone,[21] 7*H*-benz[*de*]anthracen-7-one[43] and benzo[*a*]pyrene.[34] The effects of various solvents and conditions on the recovery of benzo[*a*]pyrene, benz[*c*]acridine and 7*H*-benz[*de*]anthracen-7-one have been studied.[105]

The precision of many of the methods developed for the fluorimetric analysis of air pollutants has been reported in terms of relative standard deviation. Obviously, the precision of any of these methods is limited by the errors involved in the collection and extraction of airborne particulates.

Interference

An understanding of some of the interferences arising from the solvent, the adsorbent, or the standard or the nature of the instrument is necessary for most efficient use of the spectrophotofluorimeter.

Scatter. At very low concentrations, the scatter peak may become so intense that it interferes in the determination of the test substance. This interference is due to the scattering of the exciting light by the cuvette faces and the solution. The scatter occurs at the wavelength set on whichever monochomator is not used for spectrum scanning. The scatter peak is much larger in fluorescence spectra obtained directly from chromatograms and pherograms.

The various types of scatter which can be obtained in trace analysis are shown in the fluorescence emission spectrum of quinine in acid, Fig. 13. Many aspects of Rayleigh and Tyndall scatter[106] and of Raman scatter[104] have been discussed.

Scatter can be eliminated with suitable polarizers and fllters.[106] The major problem in most trace methods is Rayleigh and Tyndall scatter and not Raman scatter. Raman scattering is less prominent (although observable) in excitation spectra than in emission spectra because the detector response is usually chosen to be maximum at the wavelength of maximum fluorescence, in which case the scattering appearing when both monochromators are set at the same wavelength is usually much more intense than the Raman scattering intensity.[106]

However, Raman scatter can produce an appreciable distortion of the fluorescence spectrum of a strongly fluorescent substance at concentrations less than 0·1 μg/ml.

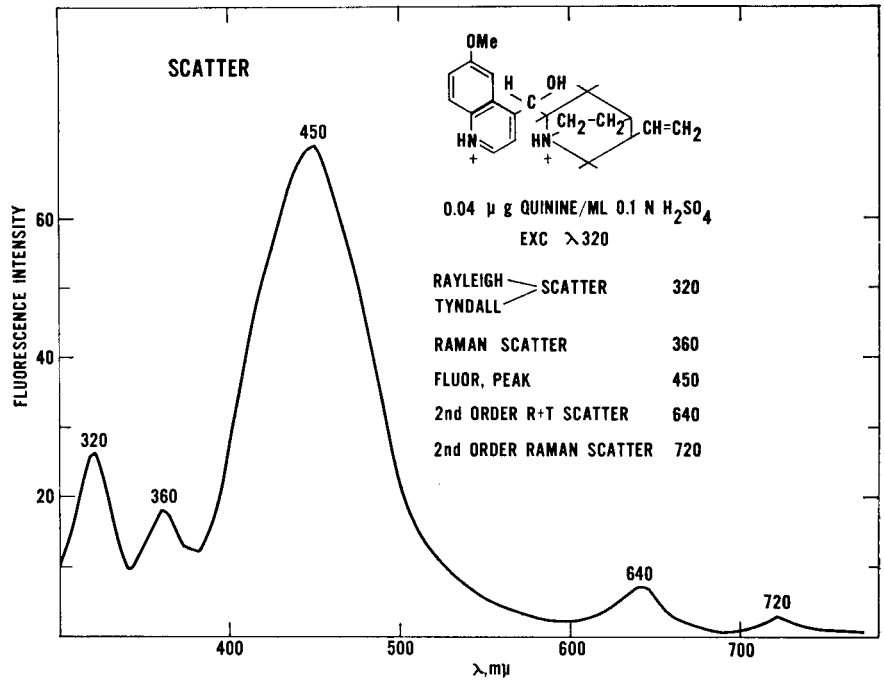


FIG. 13.—Various types of scatter in the emission spectrum of quinine sulphate.

TABLE V.—RAMAN BANDS OBSERVED IN SELECTED SOLVENTS[104]

Solvent	Frequency shifts (μ^{-1}) with exciting light at				Mean frequency shift, μ^{-1}
	248 *nm*	313 *nm*	365 *nm*	436 *nm*	
Water	0·339	0·339	0·340	0·335	0·338
Ethanol	0·292	0·292	0·293	0·290	0·292
	0·143	0·143	0·141	0·134	0·140
Cyclohexane	0·287	0·287	0·291	0·285	0·288
	0·137	0·138	0·135	0·132	0·136
Carbon tetrachloride	—	0·073	0·079	0·071	0·074
Chloroform	—	0·073	0·073	0·065	0·070
	0·301	0·301	0·301	0·304	0·302

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Parker[104] has recorded the Raman spectra of five commonly used solvents (Table V) which could affect trace analytical investigations.

Most fluorimeters and spectrophotofluorimeters use excitation at 90° to the direction of observation of fluorescence. Under these conditions, a polarizer in the emitted beam can distinguish between troublesome scattered light, which is highly polarized, and fluorescent light, which usually is negligibly polarized.

To reduce scatter, polarizers have been employed in both the exciting and the emitting beams,[106,107] but such arrangements are said to have no advantage over the use of a single polarizer in the exciting beam.[108] The reduction of scatter with a polarizer in the exciting beam oriented with the electric vector vertical and a polarizer in the emission beam with the electric vector horizontal is shown in Fig. 14.[109] In this

emission spectrum of serotonin, both first and order scatter are deleted by the polarizers.

Another method of reducing this background is use of a glass filter (placed in front of the photomultiplier tube) selected to pass only radiation of greater wavelength than that of the exciting radiation in the first order of diffraction. An example of the obliteration of scatter with a proper filter is shown in Fig. 15.[51]

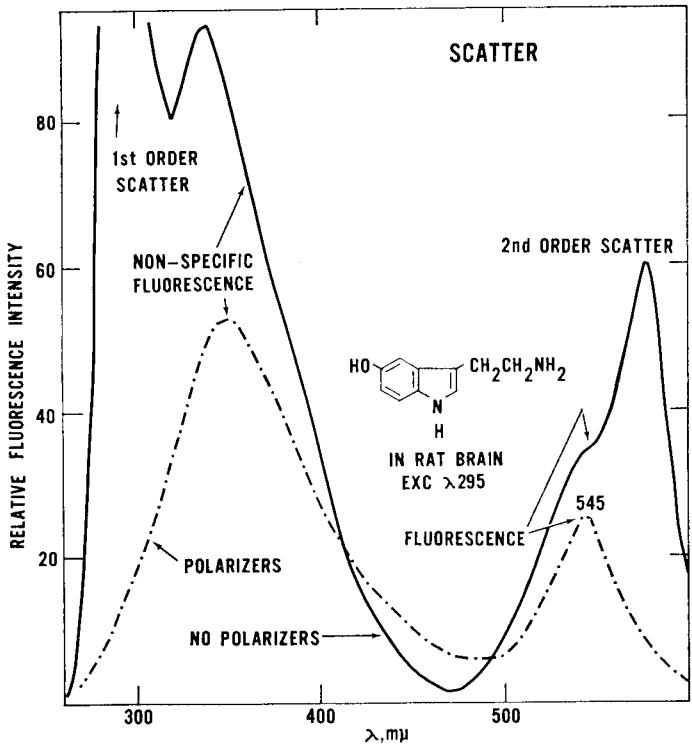

FIG. 14.—Effect of polarizer on the fluorescence emission spectrum of an extract of rat brain containing serotonin, MM = 0·001.[109] (Reprinted by permission of the copyright holders of *Anal. Biochem.*, 1965, **11**, 395.)

Impurities in solvents. One of the best ways of detecting fluorescent impurities in solvents is to use excitation at fairly short wavelength but which is still very intense-*e.g.*, 250 nm. More fluorescent molecules absorb at shorter wavelengths. Commercial solvents and reagents often contain high concentrations of fluorescent impurities.

Impurities in standards. One of the major difficulties in atmospheric trace analysis is the presence of impurities in the standards. Impurities can cause quenching effects, or in minute amounts they can result in sensitized fluorescence. Difficulties may arise if an analyst assumes that the fluorescent bands derived from the impurities are from the standard, so he should know what impurities are present in his standards.

For example, phenanthrene contains anthracene and fluorene,[110] fluorene contains carbazole,[110] chrysene contains 5*H*-benzo[*b*]carbazole,[111] benzo[*e*]pyrene contains benzo[*a*]pyrene and perylene,[2] and carbazole contains anthracene and 5*H*-benzo[*b*]-carbazole.[112] Many aza arene standards used in air pollution analysis have been shown to contain fluorescent impurities.[113]

Other background fluorescence. Cuvettes made with fused quartz fluoresce strongly in the short-wave ultraviolet range. Consequently, cuvettes of synthetic silica, which have little fluorescence in this region, should be used in trace analysis at short excitation wavelengths.

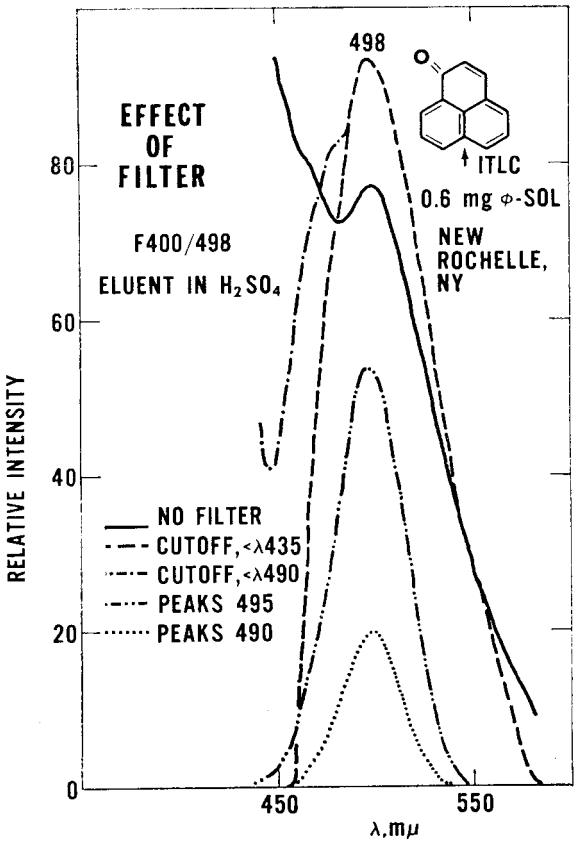

FIG. 15.—Effect of various filters on the emission spectrum of the extract of an unknown spot opposite the phenalen-1-one sot from the separation of 0·6 mg of a benzene-soluble fraction of urban airborne particulate from New Rochelle, New York, with pentane–trifluoroacetic acid (50:1 v/v) as the developer.[51]
The readings were taken at 498 nm emission with excitation at 400 nm. The extract was in 0·5 ml of concentrated sulphuric acid. (———) No filter, MM = 0·01. (– – – –) Corning Glass filter No. CS3-72, MM = 0·01. Cuts off light below 435 nm. (— · — ·) Corning Glass filter No. CS3-70, MM = 0·01. Cuts off light below 490 nm. (— · · — · ·) Filter No. 65A, MM = 0·01, Aminco Catalogue No. 47160. Transmission at 495 nm. (· · · ·) Filter No. 75, MM = 0·01, Aminco Catalogue No. 47120. Transmission at 490 nm.[59] (Reprinted by permission of the copyright holders from *J. Chromatog.*, 1968, **39**, 508.)

In the analysis of a solution, increases in the noise-to-signal ratio are due to several factors, *e.g.*, scatter, solvent impurities, background fluorescence of other components in a mixture, and background fluorescence of reagents and their products from reaction with the miscellaneous compounds in the mixture. Because of such a background, even highly sensitive procedures can lose some sensitivity. For example, in the oxidative determination of 2-deoxyribose by the J-acid, 2,4-pentanedione and dimedone procedures, the blank values were 1·8, 0·7 and 9·4 μg, respectively.[46]

These values, fairly high for such sensitive fluorimetric methods, tend to limit the sensitivity of the basic method.

Another problem in any paper chromatographic or TLC procedure is the contamination of the adsorbent by the polluted air in a room or by the fumes from a cigarette, cigar, or pipe. For example, in the direct spectrophotofluorimetric analysis of a glass-fibre paper chromatogram of a formaldehyde procursor by the J-acid procedure, cigarette fumes give a high blank value.[61] In fact, the excitation and emission spectra of the blank were identical to the analogous spectra of the test substance reaction product.

Relation between fluorescence intensity and concentration

The lower value of the useful concentration range is limited by the inherent fluorescence of the final fluorogen, by the interference of scatter at the wavelength of measurement, by the total background fluorescence, and by instrument sensitivity. The upper value is limited by quenching due to self-absorption.

Many of the spots separated on chromatograms or pherograms can be examined directly. Usually, fluorimetric scanning can then simplify the analysis. For example, in the analysis of the air pollutants phenalen-1-one and 7*H*-benz[*de*]anthracen-7-one, the calculations after scanning are based on the volume of a cone, derived from the area of the spot and the product (MM·T) of the meter multiplier and meter transmittance readings.[22] With the assumption that MM·T equals the height of the cone, the following formula is used in the calculation:

$$C_x = \frac{C_s \cdot A_x \cdot (\mathrm{MM{\cdot}T})_x}{A_s \cdot (\mathrm{MM{\cdot}T})_s}$$

where C is concentration, A is area, and the subscripts x and s refer to sample and standard respectively.

Other air pollutants that have been determined directly through fluorimetric scanning techniques include acridine,[58] benzo[*h*]quinoline,[15] benz[*c*]acridine,[15] benzo[*a*]pyrene,[69] and aza arenes.[34,69]

Three types of fluorimetric scans are possible: the filter fluorimetric type,[67,114–116] the spectrophotofluorimetric scan with each monochromator set at an appropriate wavelength for the whole scan[69] and the spectrophotofluorimetric scan with each monochromator set at an appropriate wavelength for each spot.[69]

The selective scan for dibenz[*a*, *h*]acridine separated on the cellulose thin-layer chromatogram of an alumina column chromatographic fraction of an air sample polluted with coal-tar pitch is shown in Fig. 16.[69]

The selective scan for benzo[*a*]pyrene separated on a cellulose acetate thin-layer chromatogram of an alumina column chromatographic benzpyrene fraction obtained from an urban air sample is shown in Fig. 17.[69]

ORGANIC AIR POLLUTANTS DETERMINED BY FLUORIMETRIC METHODS

Fluorescence characterization and fluorescence assay methods are available for a large number of pollutants present in the particulates of urban atmospheres and air pollution source effluents, as shown in Table VI. For most of the compounds

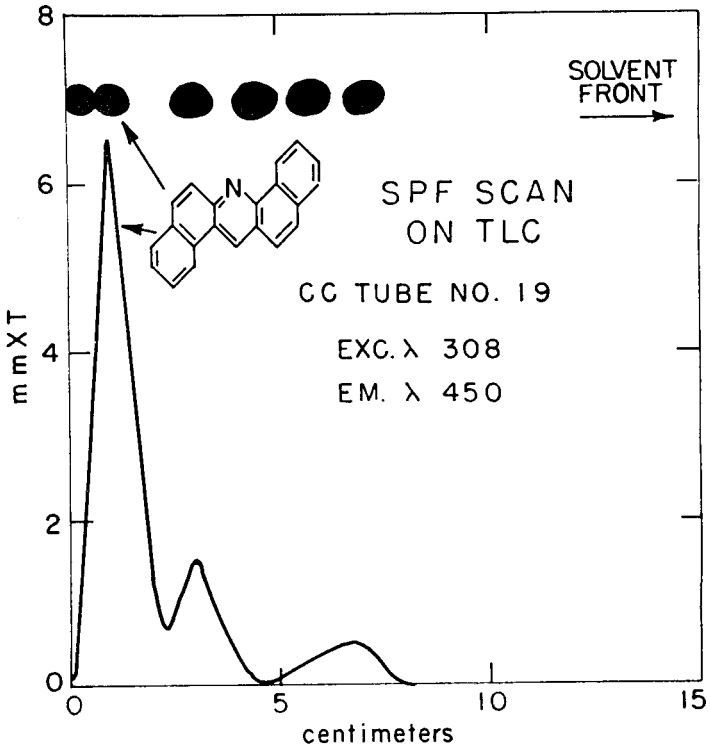

FIG. 16.—Spectrophotofluorimetric scan of a dibenz[*a*,*h*]acridine column chromatographic fraction obtained from air polluted with coal-tar pitch.
Previous to scan, sample was separated by cellulose TLC with dimethylformamide–water (35:65) as developer. Spots were treated with trifluoroacetic acid fumes before scan.[69]
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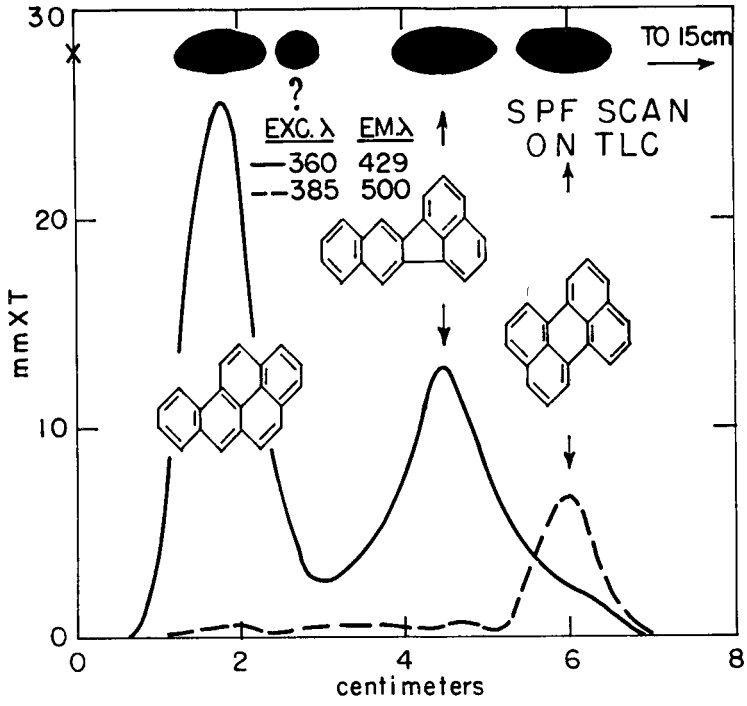

FIG. 17.—Spectrophotofluorimetric scan of a mixture containing 0·1 μg each of benzo[*a*]pyrene, benzo[*k*]fluoranthene, perylene and benzo[*e*]pyrene after separation by cellulose TLC with ethanol–toluene–water (17:4:4 v/v) as the developing solvent.[69]
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TABLE VI.—ORGANIC AIR POLLUTANTS CHARACTERIZED BY FLUORIMETRIC METHODS

Compound (assay ref.)	References	
	Urban atmospheres	Air pollution source effluents
Phenanthrene		5
Pyrene	87	5
2-Methylpyrene		5
Fluoranthene	87, 120	5
Chrysene	87	
Benz[*a*]anthracene*	87	
Benzo[*a*]pyrene* (34, 69, 84)	5, 33, 34	
Benzo[*e*]pyrene	33	
Benzo[*k*]fluoranthene	5, 120	
Perylene	33	
Anthanthrene	87	
Benzo[*g*,*h*,*i*]perylene	87	
Coronene	87	
Carbazole	25	
11*H*-Benzo[*a*]carbazole*	25	
7*H*-Benzo[*c*]carbazole*	25	
Acridine (40)		40
R-Acridine†		38
Benzo[*h*]quinoline (15)	15	38
R-Benzo[*h*]quinoline†		38
R-Benzo[*h*]quinoline†		38
Benzo[*f*]quinoline		38
R-Benzo[*f*]quinoline†		38
Phenanthridine		38
5*H*-Indeno-1,2-pyridine?		38
Benz[*a*]acridine		35, 38, 40
R-Benz[*a*]acridine*†		38
Benz[*c*]acridine (15)	15	38, 40
R-Benz[*c*]acridine†‡		38
R-Benz[*c*]acridine†‡		38
Benzo[*l*,*m*,*n*]phenanthridine		38
11*H*-Indeno[1,2-*b*]quinoline		38
Indeno[1,2,3-*i*, *j*]isoquinoline		38
Dibenz[*a*,*h*,acridine*		38
Dibenz[*a*,*j*]acridine*		38
Xanthen-9-one	26	
Phenalen-1-one (22, 43)	43	22
9-Acridanone (21)	21	
7*H*-Benz[*de*]anthracen-7-one* (22, 43, 59)	43	22
α-Glycollics (46)	46	46
1-Naphthol		23
2-Naphthol		23
2-Hydroxybiphenyl		23
4-Hydroxybiphenyl		23
2-Fluorenol	23	23
3-Fluorenol		23
2-Hydroxydibenzofuran		23
2-Hydroxycarbazole		23
Scopoletin	29	29

* Carcinogens.
† R = alkyl.
‡ Possibly carcinogenic.

in this table, the references describe the fluorescence methods of characterization; for others they describe fluorescence methods of assay. Many more compounds than those shown in Table VI have been found in urban airborne particulates[35,117] and in particulates from air pollution source effluents.[37,118,119]

Zusammenfassung—Lumineszenzerscheinungen sind bei der Analyse von Luftverunreinigungen wertvoll. Die Probleme bei der Verwendung von Anregungs- und Emissionsspektren unter verschiedenen Bedingungen werden diskutiert. Es wird gezeigt, daß Faktoren wie Lösungsmittel, pH und photochemische Effekte eine wichtige Rolle bei der fluorometrischen Analyse von Luftverunreinigungen spielen. Viele fluorometrische Methoden bei der Spurenanalyse organischer Schwebeteilchen in der Luft verwenden die direkte Messung der abgetrennten Veruneinigung auf einem Chromatogramm oder Pherogramm, Löscheffekte, Registrierung, Excimerenbildung, charge-transfer-Fluoreszenz, sensibilisierte Fluoreszenz und Photooxidation am Adsorbens oder in Lösung. Darüber hinaus werden Fluoreszenz-Analysenmethoden bezüglich Selektivität, Empfindlichkeit, Geschwindigkeit, Einfachheit, Genauigkeit, Richtigkeit, Störungen und Zusammenhang zwischen Konzentration und Fluoreszenzintensität diskutiert.

Résumé—Les phénomènes de luminescence sont valables dans l'analyse de polluants de l'air. On discute des problèmes provenant de l'emploi de spectres d'excitation et d'émission dans diverses conditions. On montre que des phénomènes tels que les influences de solvant, de pH et photochimiques jouent un rôle important dans l'analyse fluorimétrique des pollutants de l'air. Un grand nombre des méthodes fluorimétriques utilisées dans l'analyse de traces de particules organiques transportées par l'air mettent en jeu des facteurs tels que la mesure directe du polluant séparé sur un chromatogramme ou un phérogramme, des phénomènes d'extinction, l'exploration, la formation d'excimère, la fluorescence par transfert de charge, la fluorescence sensibilisée et la photo-oxydation sur adsorbant ou en solution. De plus, on discute des méthodes de détermination par fluorescence en fonction des sélectivité, sensibilité, rapidité, simplicité, exactitude, précision, interférences et relation entre concentration et intensité de fluorescence.

REFERENCES

1. C. A. Parker and W. T. Rees, *Analyst*, 1960, **85**, 587.
2. E. Sawicki, T. R. Hauser and T. W. Stanley, *Intern. J. Air Pollution*, 1960, **2**, 253.
3. C. A. Parker and W. J. Barnes, *Analyst*, 1960, **85**, 1006.
4. *Idem, ibid.*, 1960, **85**, 3.
5. E. Sawicki, T. W. Stanley, S. McPherson and M. Morgan, *Talanta*, 1966, **13**, 619.
6. J. B. Birks and D. J. Dyson, *Proc. Roy. Soc.* (*London*), 1963, **A275**, 135.
7. R. F. Chen, *Anal. Biochem.*, 1967, **19**, 374.
8. C. A. Parker, *Chem. in Britain*, 1966, 160.
9. *Idem, Proc. S.A.C. Conference*, p. 160. Heffer, London, 1965.
10. R. T. Williams and J. W. Bridges, *J. Clin. Pathol.*, 1964, **17**, 371.
11. J. S. Bellen and G. Oster, *J. Am. Chem. Soc.*, 1957, **79**, 2461.
12. G. Oster and G. K. Oster, in H. Kallmann and G. M. Spruch, Eds., *Luminescence of Organic and Inorganic Materials*, p. 186. Wiley, New York, 1962.
13. E. Sawicki and R. A. Carnes, *Mikrochim. Acta*, 1968, 148.
14. R. T. Williams, *J. Roy. Inst. Chem.*, 1959, **83**, 611.
15. E. Sawicki, H. Johnson and K. Kosinski, *Mikrochim. J.*, 1966, **10**, 72.
16. W. Bartok, P. J. Lucchesi and N. S. Snider, *J. Am. Chem. Soc.*, 1962, **84**, 1842.
17. G. Jackson and G. Porter, *Proc. Roy. Soc.* (*London*), 1961, **A260**, 13.
18. T. Foerster, *Z. Elektrochem.*, 1950, **54**, 531.
19. A. Weller and W. Urban, *Angew. Chem.*, 1954, **66**, 336.
20. H. Kokubun, *Z. Elektrochem.*, 1958, **62**, 599.
21. E. Sawicki, T. W. Stanley and W. C. Elbert, *Talanta*, 1967, **14**, 431.

22. *Idem, Mikrochim. Acta*, 1965, 1110.
23. E. Sawicki, M. Guyer, R. Schumacher, W. C. Elbert and C. R. Engel, *ibid.*, 1968, **1025**.
24. R. T. Williams and J. W. Bridges, *J. Clin. Pathol.*, 1964, **17**, 371.
25. D. F. Bender, E. Sawicki and R. M. Wilson, Jr., *Intern. J. Air Water Pollution*, 1964, **8**, 633.
26. E. Sawicki and H. Johnson, *J. Chromatog.*, 1966, **23**, 142.
27. E. Sawicki, T. R. Hauser, T. W. Stanley, W. C. Elbert and F. T. Fox, *Anal. Chem.*, 1961, **33**, 1574.
28. T. W. Stanley, E. Sawicki, H. Johnson and J. D. Pfaff, *Mikrochim. Acta*, 1965, 48.
29. E. Sawicki and C. R. Golden, *Microchem. J.*, to be published.
30. E. Sawicki and R. A. Carnes, *Mikrochim. Acta*, 1968, 148.
31. E. Sawicki, T. W. Stanley and H. Johnson, *Anal. Chem.*, 1963, **35**, 199.
32. E. Sawicki, C. R. Engel, W. C. Elbert and K. Gerloch, *Talanta*, 1968, **15**, 803.
33. E. Sawicki, W. C. Elbert, T. W. Stanley, T. R. Hauser and F. T. Fox, *Intern. J. Air Poll.*, 1960, **2**, 273.
34. E. Sawicki, T. W. Stanley, W. C. Elbert, J. Meeker and S. McPherson, *Atmos. Environ.*, 1967, **1**, 131.
35. E. Sawicki, S. P. McPherson, T. W. Stanley, J. Meeker and W. C. Elbert, *Intern. J. Air Water Poll.*, 1965, **9**, 515.
36. E. Sawicki, J. E. Meeker and M. Morgan, *Arch. Env. Health*, 1965, **11**, 773.
37. *Idem, Intern. J. Air Water Pollution*, 1965, **9**, 291.
38. E. Sawicki, T. W. Stanley and W. C. Elbert, *Occupational Health Review*, 1964, **16**, No. 3, 8.
39. E. Sawicki, T. W. Stanley, J. D. Pfaff and W. C. Elbert, *Anal. Chim. Acta*, 1964, **31**, 359.
40. E. Sawicki, T. W. Stanley and W. C. Elbert, *J. Chromatog.*, 1965, **18**, 512.
41. H. Johnson and E. Sawicki, *Talanta*, 1966, **13**, 1361.
42. E. Sawicki and H. Johnson, *Anal. Chim. Acta*, 1966, **34**, 381.
43. E. Sawicki, H. Johnson and M. Morgan, *Mikrochim. Acta*, 1967, 297.
44. E. Sawicki, T. W. Stanley and J. Pfaff, *Anal. Chim. Acta*, 1963, **28**, 156.
45. E. Sawicki, R. Schumacher and C. R. Engel, *Microchem. J.*, 1967, **12**, 377.
46. E. Sawicki and R. A. Carnes, *Mikrochim. Acta*, 1968, 602.
47. N. Mataga, K. Ezumi and T. Okada, *Mol. Physics*, 1966, **10**, 201, 203.
48. D. O. Eberle and F. A. Gunther, *J. Assoc. Offic. Agr. Chemists*, 1965, **48**, 927.
49. F. Feigl, H. E. Feigl and D. Goldstein, *J. Am. Chem. Soc.*, 1955, **77**, 4162.
50. E. Sawicki, H. Johnson and M. Morgan, *Mikrochim. Acta*, 1967, 297.
51. K. Semm and R. Fried, *Naturwissenschaften*, 1952, **39**, 326.
52. W. H. Wadman, G. J. Thomas and A. B. Pardee, *Anal. Chem.*, 1954, **26**, 1192.
53. F. Korte and H. Weitkamp, *Angew. Chem.*, 1958, **70**, 434.
54. V. G. Shore and A. B. Pardee, *Anal. Chem.*, 1956, **28**, 1479.
55. J. F. Thomas, B. D. Tebbens, E. N. Sandborn and J. M. Cripps, *Intern. J. Air Poll.*, 1960, **2**, 210.
56. E. Sawicki, T. W. Stanley and H. Johnson, *Microchem. J.*, 1964, **8**, 257.
57. E. Sawicki, T. W. Stanley and W. C. Elbert, *J. Chromatog.*, 1967, **26**, 72.
58. E. Sawicki and C. R. Engel, *Mikrochim. Acta*, 1969, 91.
59. C. R. Engel and E. Sawicki, *J. Chromatog.*, 1968, **37**, 508.
60. E. Sawicki and C. R. Engel, *Anal. Chim. Acta*, 1967, **38**, 315.
61. T. W. Stanley and E. Sawicki, *Anal. Chem.*, 1965, **37**, 938.
62. N. Seiler and J. Weichmann, *Experentia*, 1964, **20**, 559.
63. W. R. Gray and B. S. Hartley, *Biochem. J.*, 1963, **89**, 59 P.
64. E. Sawicki and H. Johnson, *Microchem. J.*, 1964, **8**, 85.
65. E. Sawicki, T. W. Stanley, W. C. Elbert and M. Morgan, *Talanta*, 1965, **12**, 605.
66. E. Bush in D. Glick, Ed., *Methods of Biochemical Analysis*, Vol. XI, pp. 149–209. Interscience, New York, 1963.
67. N. Seiler, G. Werner and M. Wiechmann, *Naturwissenschaften*, 1963, **20**, 643.
68. R. Klaus, *J. Chromatog.*, 1964, **16**, 311.
69. E. Sawicki, T. W. Stanley and W. C. Elbert, *ibid.*, 1965, **20**, 348.
70. T. Forster and K. Kasper, *Z. Elektrochem.*, 1955, **59**, 976.
71. E. L. Wehry and L. B. Rogers, *Fluorescence and Phosphorescence of Organic Molecules*, in D. M. Hercules, *Fluorescence and Phosphorescence Analysis*, pp. 113, 101. Interscience, New York, 1966.
72. J. B. Birks and L. G. Christophorou, *Nature*, 1963, **197**, 1064; *Proc. Roy. Soc.* (*London*), 1964, **A277**, 571.
73. B. Stevens, *Spectrochim. Acta*, 1962, **18**, 439.
74. J. B. Birks, D. J. Dyson and I. H. Munro, *Proc. Roy. Soc.* (*London*), 1963, **A275**, 575; 1964, **A277**, 270.

75. S. I. Kreps, M. Druin and B. Czorny, *Anal. Chem.*, 1965, **37,** 586, and references therein.
76. L. C. Mitchell, *J. Assoc. Offic. Agr. Chemists*, 1961, **44,** 643.
77. M. N. Inscoe, *Anal. Chem.*, 1964, **36,** 2505.
78. A. Berg and J. Lam, *J. Chromatog.*, 1964, **16,** 157.
79. J. Lam and A. Berg, *ibid.*, 1965, **20,** 168.
80. E. Sawicki and H. Johnson, *Mikrochim. Acta*, 1964, 435.
81. E. Sawicki, T. W. Stanley and H. Johnson, *ibid.*, 1965, 178.
82. E. Sawicki and J. Pfaff, *ibid.*, 1966, 322.
83. C. R. Engel and E. Sawicki, *J. Chromatog.*, 1967, **31,** 109.
84. E. Sawicki, T. W. Stanley, W. C. Elbert and J. D. Pfaff, *Anal. Chem.*, 1964, **36,** 497.
85. R. P. Hangebrauck, R. P. Lauch and J. E. Meeker, *J. Am. Ind. Hyg. Assoc.*, 1966, **27,** 47.
86. E. Sawicki, J. E. Meeker and M. J. Morgan, *J. Chromatog.*, 1965, **17,** 252.
87. E. Sawicki, W. C. Elbert, T. W. Stanley, T. R. Hauser and F. T. Fox, *Anal. Chem.*, 1960, **32,** 810.
88. E. Sawicki, W. C. Elbert, T. R. Hauser, F. T. Fox and T. W. Stanley, *J. Am. Ind. Hyg. Assoc.*, 1960, **21,** 443.
89. E. Sawicki, T. R. Hauser, W. C. Elbert, F. T. Fox and J. E. Meeker, *ibid.*, 1962, **23,** 137.
90. E. Sawicki, *Chemist-Analyst*, 1964, **53,** 24, 28, 56, 88.
91. P. Kotin, H. L. Falk, P. Mader and M. Thomas, *Arch. Ind. Hyg. Occup. Med.*, 1954, **9,** 153.
92. F. Goulden and M. M. Tipler, *Brit. J. Cancer*, 1949, **3,** 157.
93. B. T. Commins, *Analyst*, 1958, **83,** 386.
94. P. Wedgwood and R. L. Cooper, *ibid.*, 1953, **78,** 170.
95. A. J. Lindsey, *Anal. Chim. Acta*, 1959, **20,** 175.
96. R. L. Cooper, *Analyst*, 1954, **79,** 573.
97. R. E. Waller, *Brit. J. Cancer*, 1952, **6,** 8.
98. L. Dubois, A. Corkery and J. L. Monkman, *Intern. J. Air Pollution*, 1960, **2,** 236.
99. J. F. Thomas, B. D. Tebbens, M. Mukai and E. Sanborn, *Anal. Chem.*, 1957, **29,** 1835.
100. E. Sawicki, T. R. Hauser and S. McPherson, *Anal. Chem.*, 1962, **34,** 1460.
101. S. Belman, *Anal. Chim. Acta*, 1963, **29,** 120.
102. C. A. Parker, *Photoelectric Spectrometry Group Bulletin*, No. 13, 1961, 334.
103. C. A. Parker and W. T. Rees, *Analyst*, 1960, **85,** 587.
104. C. A. Parker, *ibid.*, 1959, **84,** 446.
105. T. W. Stanley, J. E. Meeker and M. J. Morgan, *Env. Sci. Technol.*, 1967, **1,** 927.
106. J. M. Price, M. Kaihara and H. K. Howerton, *Appl. Optics*, 1962, **1,** 521.
107. R. P. Davis and M. Canessa-Fischer, *Anal. Biochem.*, 1965, **10,** 325.
108. R. F. Chen, *Anal. Biochem.*, 1966, **12,** 497.
109. P. A. Adie and M. R. Hughes, *ibid.*, 1965, **11,** 395.
110. C. A. Parker, *Proc. S.A.C. Conference*, p. 160. Heffer, London, 1965.
111. D. F. Bender, E. Sawicki and R. M. Wilson, Jr., *Anal. Chem.*, 1964, **36,** 1011.
112. M. Furusawa, T. Takeuchi, K. Ogura and A. Matsushita, *Kogyo Kagaku Zasshi*, 1966, **69,** 629.
113. C. R. Engel and E. Sawicki, *J. Chromatog.*, 1967, **31,** 109.
114. W. M. Connors and W. K. Boak, *ibid.*, 1964, **16,** 243.
115. N. Seiler and M. Wiechmann, *Z. Anal. Chem.*, 1966, **220,** 109.
116. G. Pataki and E. Strasky, *Chimia*, 1966, **20,** 361.
117. E. Sawicki, *Arch. Envir. Health*, 1967, **14,** 46.
118. M. J. Lyons, *Comparison of Aromatic Polycyclic Hydrocarbons from Gasoline Engine and Diesel Engine Exhausts, General Atmospheric Dust and Cigarette Smoke Condensate*, in E. Sawicki and K. Cassel, *Analysis of Carcinogenic Air Pollutants*, p. 193. National Cancer Institute Monograph No. 9, U.S. Govt. Printing Office, Washington, 1962.
119. D. Hoffman and E. L. Wynder, *Analytical and Biological Studies on Gasoline Engine Exhaust*, in E. Sawicki and K. Cassel, *Analysis of Carcinogenic Air Pollutants*, p. 193. National Cancer Institute Monograph No. 9, U.S. Govt. Printing Office, Washington, 1962.
120. E. Sawicki, T. W. Stanley and W. C. Elbert, *Talanta*, 1964, **11,** 1433.

Talanta, 1969, Vol. 16, pp. 1267 to 1275. Pergamon Press. Printed in Northern Ireland

CATALYTIC TITRANTS AND CATALYTIC INDICATION OF END-POINTS

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Summary—Methods of analysis based on rate measurement or exploiting properties of systems not at equilibrium are becoming important in analytical chemistry. Kinetic and equilibrium considerations of catalytic titrants and catalytic end-point detection are presented and discussed. Applications of the approach to the determination of major and trace components of organic and inorganic species are included and discussed.

ANALYTICAL methods based on kinetic considerations and mechanistic studies of chemical reactions of analytical interest are attracting increasing attention.[1] The present paper comments on the application of some kinetic concepts, combined with equilibrium considerations, to a novel approach to end-point detection.

TITRIMETIC ANALYSIS AND CATALYTIC END-POINT DETECTION

An almost neglected end-point detection method (termed "catalytic" or "catalimetric"[1–7]) can be used when the titrant is a catalyst for a given reaction, the general scheme being

$$\text{Titrant} + \text{Sample} \longrightarrow \text{Products} \tag{1}$$

$$\text{Indicator reaction} \xrightarrow{\text{Excess of titrant (catalyst)}} \text{Products} \tag{2}$$

If the titrant solution contains a component such as a metallic cation, the method may be useful for the determination of some anions, either by complexation or precipitation, provided a suitable "indicator" reaction (that which is catalysed) is found. Moreover, due to the small concentration of catalyst generally required to accelerate the indicator reaction, the method is applicable to dilute solutions, and can be of particular value in trace determinations.[3] Metal ions are well known as catalysts for many reactions, particularly of the redox type. Recent reviews[8–10] have described the wide variety of reactions available and the main difficulty is the selection of a titrant and an indicator reaction.

The indicator reaction takes place at a rate which depends on the excess of titrant added. The end-point, after reaction (1) has been (in general, stoichiometrically) completed, could be located from plots of observed rate constants *vs.* volume of titrant added,[1,11] directly by extrapolation of the linear segments of a recorded titration curve,[3] or from plots derived from the kinetics of an indicator reaction.[3]

A simple and general example can be considered because most indicator reactions useful for analytical end-point detection involve a single reactant concentration variable in the rate expression. Representing the general reaction as:

$$R \xrightarrow{c} P$$

where R is the reactant, P the product(s), and c the catalyst (titrant), and assuming that the change of concentration of R is the monitored variable, the general differential equation may be written as:

$$-\frac{d[R]}{dt} = k[R]^n.$$

Experimental conditions can usually be chosen to make $n = 1$ (first-order reaction). The dependence of rate on catalyst concentration may be expressed in most cases as $k = k_1 + k_2 \cdot C_c$; C_c is the analytical concentration of catalyst, k_1 and k_2 characterize the rate of the uncatalysed and catalysed reactions respectively. Generally $k = k_1 = 0$, near zero reaction time.

If the catalyst is added at a constant rate, so that dC_c/dt is constant, the following expression can be written:

$$-\frac{d[R]}{dC_c} \propto \frac{d[R]}{dt} = k_1[R] + k_2 \cdot C_c[R]$$

and hence:

$$-\frac{d[R]}{dC_c} = k_1'[R] + k_2' \cdot C_c[R]$$

and rearranging:

$$-\frac{d[R]}{[R]} = k_1' \, dC_c + k_2' \cdot C_c \cdot dC_c$$

Since in most cases $k_1' \cdot dC_c \ll k_2' \cdot C_c \cdot dC_c$, *i.e.*, the uncatalysed reaction does not occur at an appreciable rate, simplifying, integrating and rearranging again gives:

$$-d(\ln[R]) = k_2'' \, dC_c^2$$

or simply

$$\log [R] \propto C_c^2$$

Thus, a plot of log of reactant concentration *vs.* C_c^2 should consist of two straight-line segments crossing at the end-point. A recently reported example is the titration of cyanide ion with copper(II), with the aid of the ascorbic acid–oxygen indicator reaction.[3]

If the uncatalysed reaction proceeds at an appreciable rate:

$$\log [R] \propto (C_c + C_c^2)$$

then since $C_c \gg C_c^2$, a plot of log [R] *vs.* C_c may be used to locate the end-point.

A similar treatment could be applied to multi-order reactions ($n > 1$). If the reaction, for instance, were second-order in reactant, then rate $\propto [R]^2$ and if C_R is the initial analytical concentration and [R] the instantaneous concentration of reactant,

$$-\frac{d[R]}{dt} = k\{C_R - (C_R - [R])\}^2 = k[R]^2.$$

If, as before, the dependence on catalyst concentration can be expressed by $k = k_1 + k_2 . C_c$, then

$$-\frac{d[R]}{[R]^2} = \{k_1' + k_2' . C_c\} dC_c$$

and, as before, if the uncatalysed reaction does not occur at an appreciable rate,

$$-d(1/[R]) = k_2'' . dC_c^2$$

and the end-point could be located from linear plots of $1/[R]$ *vs.* C_c^2. If the uncatalysed reaction, however, occurs at appreciable rate, then $1/[R] \propto C_c^2$; $C_c \gg C_c^2$; and the end-point could be located from the linear plot of $1/[R]$ *vs.* C_c.

INDICATOR REACTIONS

Considerations in the selection of the titrant-indicator system include a high stability constant for reaction (1), or small solubility product in case of precipitation, as well as a sensitive reaction which can only take place at a noticeable rate under the catalytic effect of a very small excess of titrant, and in the same experimental environment as reaction (1). Side-reactions between titrant or species to be titrated, and any of the indicator reaction components (either reactants or products) must be considered. The factors to be taken into account in complexation titrations with an indicator reaction involving electron exchange have been discussed recently.[12]

A few examples of indicator reactions are presented here to illustrate practical cases of catalytic end-point detection.

The indicator reaction cerium(IV)–arsenic(III)

Trace amounts of iodide ion (10^{-6}–$10^{-7}M$) catalyse the slow reaction between cerium(IV) and arsenic(III),

$$2\ \text{Ce(IV)} + \text{As(III)} \leftrightarrows 2\ \text{Ce(III)} + \text{As(V)}. \qquad (3)$$

Reaction (3), which takes place in acidic medium ($1M$ sulphuric acid), is used for the determination of protein-bound iodine in blood.[14] The rate is followed photometrically by measuring at 370–420 nm the fading of the yellow colour due to Ce(IV). Within a limited range of reaction conditions the reaction is first-order with respect to catalyst and to reactants. A probable mechanism for the reaction has been given:[15]

$$\text{Ce(IV)} + I^- \rightarrow I + \text{Ce(III)}$$
$$2\ I \rightarrow I_2$$
$$I_2 + H_2O \rightleftarrows HIO + I^- + H^+$$
$$H_3AsO_3 + HIO \rightarrow H_3AsO_4 + I^- + H^+.$$

Chloride ions seem to be needed to stabilize unipositive iodine and to avoid a side-reaction leading to iodate which is ineffective as catalyst.[16] Traces of mercury(II) have been determined by using this reaction in a non-titrimetric method.[17]

Yatsimirskii and Fedorova,[11] introducing what was perhaps the first application of catalytic end-point detection, reported a catalytic "titrimetric" estimation of silver ion at a concentration of $10^{-6}M$. They performed a simulated titration by following, photometrically, the course of the reaction in samples containing equal amounts of silver ion and increasing exesses of titrant (iodide catalyst). Since the reaction is first-order with respect to catalyst, plots of log absorbance *vs.* time yield straight

lines with slopes directly proportional to the catalyst concentration. As long as silver is in excess, the addition of iodide only results in an increase in the silver iodide formed (K_{sp} for AgI at 25° = $8{\cdot}3 \times 10^{-17}$). Up to the equivalence point the free iodide concentration will be in the vicinity of $9 \times 10^{-9}M$, below the concentration required for a noticeable rate of the indicator reaction. Because the catalyst concentration remains small, and proportional to the reciprocal of the equilibrium concentration of silver ion until the equivalence point is reached, the slope of log absorbance *vs.* time will remain constant. As soon as an excess of catalyst is present in the system, the slope of the first-order plot varies dramatically with increasing catalyst concentration. The slopes of the first-order plots are plotted against the volume of titrant added. The intercept of the lines representing the "uncatalysed" and catalysed reactions gives the end-point and should correspond theoretically to the equilibrium concentration of iodide arising from the solubility of silver iodide in the medium and at the temperature used. The value found by those workers ($[I^-] = 10^{-8}M$) compares well with the solubility of silver iodide at temperatures close to 25°. The authors claimed an error for the determination of $\pm 1\%$. This will include a bias of 0.2% due to the solubility of silver iodide.*

Recently, the same authors[18] have reported a method for the catalytic titrimetric determination of Pd(II) at the $10^{-6}M$ level, using the same indicator reaction and titrant. They claim a relative error of $\pm 1{\cdot}8\%$. All other methods available for the determination of traces of palladium(II) in aqueous solution are limited to the range 10^{-2}–$10^{-5}M$ with relative errors of ± 2–4%. The same indicator reaction and $0{\cdot}10M$ iodide solution as titrant have been used by Weisz and Klockow[6] to titrate 50–108 mg of silver ion with a relative error of $\pm 0{\cdot}25\%$, and 60–100 mg of mercury(II) with a relative error of $\pm 0{\cdot}15\%$. This application shows the method working at the macro-level and adds the interesting variation of use of potentiometry. Since mercury(II) is known to give very stable complexes with sulphur-containing ligands, this same iodide-catalysed reaction has been explored for the detection and determination of such complexing agents.[19] To allow better complexation conditions this application was studied at sulphuric acid concentrations of 0·1–0·2M. Ligands which have been detected or determined include mercaptoacetic acid, 2-aminoethylthiol, thioacetamide, and dithio-oxamide. The experimental conditions used[18] were pH $\sim 0{\cdot}3$ and analytical concentrations of $7{\cdot}7 \times 10^{-8}M$ iodide and $1{\cdot}1_5 \times 10^{-7}M$ mercury(II). The range of ligand concentration reported as useful for detection-determination purposes is 10^{-5}–$10^{-6}M$.

The indicator reaction cerium(IV)–antimony(III)–ferroin[7]

This reaction has been used for the determination of microamounts of iodide as catalyst and silver or mercury(II) which act as inhibitors, with a relative error of 1% at the 10-μg level.

The indicator reaction ascorbic acid–oxygen

The oxidation of ascorbic acid (H_2A) by dissolved oxygen in an aqueous medium can be followed photometrically by monitoring the absorbance of the monoprotonated

* %Titration error = 100{([Ag] at the end-point)/C_{Ag}} where [Ag] = equilibrium silver concentration, C_{Ag} = analytical silver concentration. Since a silver concentration of $4 \times 10^{-6}M$ was used in the determinations,[1] the error due to solubility and ionization of AgI is $100(9{\cdot}12 \times 10^{-9}/4{\cdot}00 \times 10^{-6}) = 0{\cdot}23\%$.

species (HA^-) at ~265 nm. The reaction is suitable for the detection and determination of traces of copper(II), which acts as a catalyst, and for the catalytic titration of ligands capable of forming very strong complexes with Cu(II) or Cu(I).[3,12,20]

The catalysed reaction is faster at lower hydrogen ion concentrations, so a wide pH range is available for selective titration of different ligands. Cyanide ions have been titrated at the $10^{-7}M$ level with good precision (pH 10·5),[3] and 10^{-6}–$10^{-5}M$ EDTA at pH 6·9.[20] The concentrations of titrant [copper(II) perchlorate] were $n \times 10^{-5}M$ and $n \times 10^{-3}M$, respectively, chosen to compensate for the pH effect on the rate constant.

At pH 6·9 $[HA^-]$ is effectively the analytical concentration of ascorbic acid, C_{H_2A}, and the rate expression may then be written as:

$$-\frac{d[HA^-]}{dt} = k_1 C_{H_2A} + k_2 C_{H_2A}[Cu(II)]$$

where k_1 and k_2 are constants characterizing the uncatalysed and catalysed reaction respectively.

At pH 10·5 or above, however, the concentration of HA^- is in effect given by:

$$[HA^-] = \frac{K_{a1}[H^+]C_{H_2A}}{K_{a1}K_{a2} + K_{a1}[H^+]}$$

and the rate expression is better represented as:

$$-\frac{d[HA^-]}{dt} = k_1 \frac{K_{a1}[H^+]C_{H_2A}}{K_{a1}K_{a2} + K_{a1}[H^+]} + k_2 \frac{K_{a1}[H^+]C_{H_2A}[Cu(II)]}{K_{a1}K_{a2} + K_{a1}[H^+]} \quad (5)$$

where K_{a1} and K_{a2} are the first and second ionization constants of ascorbic acid ($10^{-4.04}$ and $10^{-11.34}$ respectively).

At pH 6·9, and the analytical concentration of copper(II) used experimentally,[20] Cu(II) would be present mainly as Cu^{2+} and $Cu(OH)^+$, since $K = 10^{6.66}$ for $Cu^{2+} + OH^- \rightleftarrows CuOH^+$ and the total concentration is below that ($10^{-4}M$) corresponding to the solubility[21] of $Cu(OH)_2$:

$$Cu(OH)_2 \rightleftarrows Cu^{2+} + 2\ OH^- \qquad {}^{+}K_{sp} = 10^{-18.2}$$

whence

$$\log [Cu^{2+}] = 9{\cdot}8 - 2\ pH = -4 \text{ at pH } 6{\cdot}9$$

Under these conditions, a complex formed by the HA^- and Cu(II) may well take part in the autoxidation mechanism, and has been proposed to explain catalysis in the pH range 2–5·5.[22]

The considerable increase in catalytic effect at pH > 7, however, appears to be more complicated. Hydroxyl ions are predominant, thus the concentration of Cu^{2+} is considerably decreased. The increased catalytic effect may be due to the pH effect shown in equation (5) and/or to complexation by A^{2-}.

LIGAND-EXCHANGE REACTIONS AS INDICATOR SYSTEMS

Indicator reactions are not limited to electron-exchange reactions. Ligand-exchange reactions, for instance, offer attractive systems for end-point detection. Although no direct titrimetric application has been reported, the use of ligand-exchange reactions appears to be practicable.

Margerum and Olson[23] first suggested the approach and its analytical potential in 1963. Since then, Margerum and co-workers have reported some applications for the determination of traces of metal ions and some strong complexing agents.[24,25]

References 23 and 24 provide interesting and useful considerations of equilibrium and kinetic concepts. The indicator reaction in both cases is:

$$NiL_T^{2+} + CuL_E^{2-} \rightarrow CuL_T^{2+} + NiL_E^{2-} \qquad (6)$$

where L_T is triethylenetetramine $H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$, and L_E^{4-} is the EDTA anion $(OOCCH_2)_2NCH_2CH_2N(CH_2COO)_2^{4-}$.

From the facts that (*a*) the rate of the indicator reaction is much faster than the rate of dissociation of either NiL_T^{2+} or CuL_E^{2-}, (*b*) the rates of attack of Ni^{2+} on CuL_E^{2-} or Cu^{2+} on NiL_T^{2+} are both too slow to contribute significantly to the reaction rate, owing to the low concentration of metal ion, (*c*) the rate of attack of L_E^{4-} on NiL_T^{2+} is known to be fast (even at low ligand concentration), and the assumption that the rate of attack of L_T on CuL_E^{2-} is also fast, Olson and Margerum[23] proposed the following mechanism for reaction:

chain-initiating step: $NiL_T^{2+} \rightleftarrows Ni^{2+} + L_T$

chain-propagating steps: $L_T + CuL_E^{2-} \rightleftarrows CuL_T^{2+} + L_E^{4-}$

$L_E^{4-} + NiL_T^{2+} \rightarrow NiL_E^{2-} + L_T$

chain-terminating step: $L_E^{4-} + Ni^{2+} \rightarrow NiL_E^{2-}$.

Using this model, it is easy to infer that the analytical applications rest on the possibility of determining metal ions which complex either L_T or L_E^{4-} strongly. A third ligand, which could compete favourably with L_E^{4-} or L_T for complexation of Cu^{2+} or Ni^{2+}, could also be determined.

By combination of the $NiL_T^{2+} + CuL_E^{2-}$ reaction with the following set of reactions:

$$2\ Co(CN)_5^{3-} + O_2 \rightarrow (CN)_5CoO_2Co(CN)_5^{6-}$$

$$Co(CN)_5^{3-} + Co(NH_3)_5Br^{2+} \rightarrow Co(CN)_5Br^{3-} + Co(NH_3)_5H_2O^{2+}$$

$$Co(NH_3)_5H_2O^{2+} + L_E^{4-} \rightarrow CoL_E^{2-} + 5\ NH_3$$

as little as 10^{-8} mole of oxygen in 10 ml of water has been determined,[26] and it should be possible to detect as little as 10^{-9} mole of oxygen in 10 ml of water, which compares extremely well with the usual limit of commercially available oxygen meters, which is about 30 times greater. The kinetic method, however, might be subject to more interferences and be more sensitive to experimental conditions.

HYDROXYL-ION CATALYSED INDICATOR REACTIONS AND CALORIMETRIC-CATALYTIC END-POINT INDICATION

Most of the catalytic indicator reactions reported in the literature have been followed spectrophotometrically. In a few cases other means of sensing have been applied such as potentiometry.[6]

The titration of 2,6-disubstituted phenols, keto-enols, imides, and traces of acid, with non-aqueous alkaline solutions,[27] and the titration of tertiary amines and salts of organic acids in acetic acid[28] are examples of determinations using calorimetric catalytic end-point detections.

Titrations involving non-aqueous (other than acetone) alkaline solutions give titration curves with rather little change in slope in the vicinity of the equivalence point. However, if an acidic species dissolved in acetone is titrated with hydroxyl

ions in an ionizing non-aqueous solvent other than acetone, the "neutralization" reaction takes place first, and the first excess of OH^- catalyses the indicator reaction (formation of diacetone alcohol) which takes place with considerable release of heat.

The indicator reaction and the proposed mechanism[27] responsible for the end-point detection are:

reaction $$2\ CH_3COCH_3 \rightarrow CH_3COCH_2COH(CH_3)_2$$

echanism: $$CH_3COCH_3 + OH^- \rightleftarrows CH_3COCH_2^-$$
$$CH_3COCH_2^- + CH_3COCH_3 \rightleftarrows CH_3COCH_2C(O^-)(CH_3)_2 \qquad (7)$$
$$CH_3COCH_2(O^-)(CH_3)_2 + H_2O \rightleftarrows CH_3COCH_2COH(CH_3)_2 + OH^-$$

Reaction (7) has been reported to be slow[29] and the acid-base reaction can take place before any catalytic action of OH^- can be exerted on the slower exothermic condensation reaction giving 4-hydroxy-4-methyl-2-pentanone (diacetone alchol).

Acetic anhydride is commonly used to remove water from glacial acetic acid used as solvent in acid-base titrations; Vajgand and Gaál[28] have shown, however, that with glacial acetic acid containing approximately 2% water and 8% acetic anhydride, tertiary amines can conveniently be determined enthalpimetrically. The indicator reaction is:

$$(CH_3CO)_2O + H_2O \rightarrow 2\ CH_3COOH$$

but the mechanism is not yet definitely known. An excess of perchloric acid present after the stoichiometric reaction:

$$\text{Organic base} + HClO_4 \rightleftarrows (\text{Protonated base})^+ + ClO_4^-$$

has taken place, will catalyse the indicator reaction. The authors have reported the titration of $2{\cdot}5\text{–}5{\cdot}7 \times 10^{-2}N$ base and pointed out that the method appears to be more accurate and reproducible than the potentiometric determination, particularly in those cases when the titrated substance gives a precipitate with perchloric acid.

ENZYME-CATALYSED REACTIONS AS INDICATOR REACTIONS

Although no catalytic titrations, as defined here, have been reported for enzyme-catalysed reactions, it is well known that some metal ions act as enzyme activators and many others behave as inhibitors, slowing down the rate of the reaction. Little attention has been paid to the use of these systems in the determination of metal and non-metal ions in solution. The general approach to catalytic end-point indication, however, should be worth exploring with systems of this type.

An example involves the metal-activated enzyme isocitric dehydrogenase (ICD) and the following reaction:

$$\text{TPN} + \text{DL-isocitrate} \xrightarrow[\text{ICD}]{\text{metal activator}} CO_2 + \text{TPNH} + \alpha\text{-KG} \qquad (8)$$

where TPN is triphosphopyridine nucleotide, TPNH the reduced form of TPN, and α-KG α-ketoglutarate.

Only Mg^{2+} and Mn^{2+} have been found to activate this reaction[30] and many other ions [Ag, Hg(II), Ba, Ca, Al, Sr, Pb, and Cu(II)] have been found to act as inhibitors.[31]

If reaction (8) is coupled with a reaction of the type

$$\text{TPNH} + \underset{\text{(coloured)}}{\text{oxidized form of a dye}} \rightarrow \text{TPN} + \underset{\text{(colourless)}}{\text{reduced form of a dye}}$$

the extent of reaction can be monitored, and the system may be used for the detection and determination of cations and anions in solution. The extent of reaction may also be followed by measuring the absorbance of the solution at 340 nm, the wavelength at which TPNH absorbs. This approach has been recently applied in following the overall reaction:[32]

$$\text{Isocitric acid} + \text{TPN} \xrightarrow[\text{metal activator}]{\text{ICD}} \alpha\text{-KG} + \text{TPNH} + CO_2$$

which has been used for trace metal analysis by a simulated titration procedure[32] with EDTA as titrant.

The determination of metal ions which inhibit the oxidation of glucose by molecular oxygen in presence of glucose-oxidase has been reported recently.[33] The reaction system is

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$
$$H_2O_2 + o\text{-dianisidine} \xrightarrow{\text{horseradish peroxidase}} H_2O + \text{oxidized } o\text{-anisidine}$$

The oxidized form of *o*-anisidine absorbs at 440 nm and offers a way of following the reaction. Results for Ag, Hg(II), and Pb(II) are reported.[33] Silver could be detected at levels of $10^{-6}M$. The system is less sensitive for mercury and considerably less for lead under the reported experimental conditions. The lack of sensitivity seems to be due to masking by buffer components.

CONCLUSIONS

The reactions chosen for presentation in this article provide good examples of the usefulness of catalytic end-point indication for the titrimetric determination of inorganic and organic species in solution and in a relatively wide range of concentration. This outline is presented in the hope that it will encourage further consideration of this approach in the development of analytical methods as well as in the study of kinetics, mechanism, and thermodynamics of chemical reactions of analytical interest.

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Zusammenfassung—Analysenmethoden, die auf Geschwindigkeitsmessungen beruhen oder Eigenschaften von nicht im Gleichgewicht befindlichen Systemen ausnutzen, gewinnen in der analytischen Chemie an Bedeutung. Kinetik und Gleichgewicht katalytischer Titranten und katalytischer Endpunktsanzeige werden erörtert. Anwendungen auf die Bestimmung von Haupt- und Spurenbestandteilen organischer und anorganischer Spezies werden ebenfalls diskutiert.

Résumé—Les méthodes d'analyse basées sur la mesure de vitesse ou l'exploitation de propriétés de systèmes qui ne sont pas à l'équilibre deviennent importantes en chimie analytique. On présente des considérations de cinétique et d'équilibre sur les produits de titrage catalytiques et la détection catalytique du point de fin de dosage et en discute. Des applications sur l'accès à la détermination de composants majeurs et à l'état de traces d'espèces organiques et minérales sont incluses et discutées.

REFERENCES

1. K. B. Yatsimirskii, *Kinetic Methods of Analysis*, Pergamon, Oxford, 1966; H. B. Mark, Jr. and G. A. Rechnitz, *Kinetics in Analytical Chemistry*, Interscience, New York, 1968.

2. T. I. Fedorova and K. B. Yatsimirskii, *Zh. Analit. Khim.*, 1967, **22**, 283.
3. H. A. Mottola and H. Freiser, *Anal. Chem.*, 1968, **40**, 1266.
4. H. Weisz and U. Muschelknautz, *Z. Anal. Chem.*, 1966, **215**, 17.
5. H. Weisz and T. Janjić, *ibid.*, 1967, **227**, 1.
6. H. Weisz and D. Klockow, *ibid.*, 1967, **232**, 321.
7. J. Bognár and S. Sárosi, *Mikrochim. Acta*, 1963, 1072; 1965, 1004; 1966, 534.
8. G. A. Rechnitz, *Anal. Chem.*, 1964, **36**, 453R; 1966, **38**, 513R; 1968, **40**, 455R.
9. J. G. Hanna and S. Siggia, *J. Pharm. Sci.*, 1966, **55**, 541.
10. Z. Gregorowicz and T. Suwińska, *Chem. Analit. (Warsaw)*, 1966, **11**, 3.
11. K. B. Yatsmirskii and T. I. Fedorova, *Proc. Acad. Sci. USSR*, 1962, **143**, 143.
12. H. A. Mottola, M. S. Haro and H. Freiser, *Anal. Chem.*, 1968, **40**, 1263.
13. E. B. Sandell and I. M. Kolthoff, *J. Am. Chem. Soc.*, 1934, **56**, 1426; *Mikrochim. Acta*, 1937, **1**, 9.
14. A. L. Chaney, *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 179.
15. W. C. Bray, *Chem. Rev.*, 1932, **10**, 161.
16. H. A. Laitinen, *Chemical Analysis*, p. 461. McGraw-Hill, New York, 1960.
17. M. T. Beck and F. Gaizer, *Michrochem. J.*, 1965, **9**, 316.
18. T. J. Fedorova and K. B. Yatsmirskii, *Zh. Analit. Khim.*, 1967, **22**, 283.
19. G. Miller and H. Freiser, personal communication.
20. H. A. Mottola, K. Muller and H. Freiser, unpublished results.
21. L. G. Sillén and A. E. Martell, *Stability Constants of Metal Ion Complexes*, Chemical Society, London, 1964.
22. M. M. Taqui Khan and A. E. Martell, *J. Am. Chem. Soc.*, 1967, **89**, 4176.
23. D. C. Olson and D. W. Margerum, *ibid*, 1963, **85**, 297.
24. D. W. Margerum and R. K. Steinhaus, *Anal. Chem.*, 1965, **37**, 222.
25. R. H. Stehl, D. W. Margerum and J. J. Latterell, *ibid.*, 1967, **39**, 1346.
26. D. W. Margerum and R. H. Stehl, *ibid.*, 1967, **39**, 1351.
27. G. A. Vaughan and J. J. Swithenbank, *Analyst*, 1965, **90**, 594.
28. V. J. Vajgand and F. F. Gaál, *Talanta*, 1967, **14**, 345.
29. P. Sykes, *A Guide Book to Mechanisms in Organic Chemistry*, 5th Ed., p. 158. Longmans, London, 1963.
30. G. Siebert, M. Carsiotis and G. W. E. Plaut, *J. Biol. Chem.*, 1957, **226**, 977.
31. D. N. Kramer, P. L. Cannon and G. G. Guilbault, *Anal. Chem.*, 1962, **34**, 842.
32. B. Kratochvil, S. L. Boyer and G. P. Hicks, *ibid.*, 1967, **39**, 45.
33. E. C. Torren, Jr. and F. J. Burger, *Mikrochim. Acta*, 1968, 538.

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ACIDITY OF SEVERAL CHROMOTROPIC ACID AZO DERIVATIVES

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Summary—Both potentiometric and spectrophotometric methods have been used for the determination of the stability constants of hydrogen complexes of 4,5-dihydroxynaphthalene-2,7-disulphonic acid (Chromotropic Acid, CA), 3,6-bis(phenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Azo III, A III), 3,6-bis(2′-sulphophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Sulphonazo III, SA III), 3,6-bis(4′-methyl-2′-sulphophenylazo)-4,5-dihydroxynaphthalene 2,7-disulphonic acid (Dimethylsulphonazo III, DMSA III), 3-(4′-chloro-2′-phosphonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Chlorophosphonazo I, CPA I), 3,6-bis(4′-chloro-2′-phosphonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Chlorophosphonazo III, CPA III), 3-(2′-arsonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Arsenazo I, AA I) and 3,6-bis(2′-arsonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Arsenazo III, AA III).

MANY 4,5-dihydroxy-naphthalene-2,7-disulphonic acid azo derivatives are used as analytical reagents in spectrophotometry and complexometry.[1,2] The unusual behaviour of these reagents has promoted the study of composition, stereochemistry and stability of their complexes.[3–21] Recently an attempt was made to explain the relation between colour and complexing properties by means of molecular orbital theory.[22–24] Unfortunately, these studies have so far had limited success because of incorrect assumptions about the acidity and stereochemistry of the reagents. Many authors have used impure material, inconvenient calculation methods, inadequate spectrophotometers or pH-meters, and have not considered the extremely strong bonding of the last proton of chromotropic acid hydroxyl groups. These are some of the reasons why so many wrong values of stability constants of chromotropic acid azo derivatives have been previously published.

The most probable stereochemistry of metal complexes of chromotropic acid azo derivatives has been discussed recently.[2,25] The acidities of 4,5-dihydroxynaphthalene-2,7-disulphonic acid and 3-phenyl-azo-4,5-dihydroxy-naphthalene-2,7-disulphonic acid (Azo I, A I) were investigated by Schwarzenbach and co-workers in 1951.[26,27] They found an extremely low acidity (pH $>$ 14) for the dissociation of the last proton. Despite this many authors suppose this acidity should be about pH 11–12.[15,20,21]

Because of these discrepancies we have reinvestigated the acidity properties of several important reagents. Our results are contained in this paper.

EXPERIMENTAL

Apparatus

All photometric measurements were made with a double beam recording spectrophotometer Unicam SP800A, with 10-mm quartz cells.

An Orion Model 801 pH meter, with combined glass and calcomel electrode, was used for pH measurement.

Reagents

Aqueous $1{\cdot}00 \times 10^{-4}M$ stock solutions of individual reagents were used throughout. The purity of all reagents used was controlled by elemental analysis and paper chromatography (Whatman paper No. 1, elution system of 2% aqueous ammonia saturated with isobutanol). Since the free acids are the best form for elemental analyses and for potentiometric titration, the sodium salts were converted into the free acids by passing their aqueous solutions through a column of a strongly acidic cation exchanger, *e.g.*, Dowex 50WX8. The results of analysis and the corresponding R_F values are collected in Table 1. All reagents used were chromatographically pure.

TABLE I.—ELEMENTAL ANALYSIS AND R_F VALUES OF INDIVIDUAL REAGENTS

Reagent	R_F	M.W.	N,%		S,%	
			Found	Calc.	Found	Calc.
A III	0·35	528·5	10·4	10·60	12·3	12·13
SA III	0·47	688·7	8·1	8·14	18·5	18·62
DMSA III	0·38	716·7	7·8	7·82	17·8	17·90
CPA I	0·45	538·9	5·0	5·20	11·8	11·90
CPA III	0·65	757·4	7·3	7·40	8·4	8·47
AA I	0·84	548·3	5·0	5·11	11·6	11·70
AA III	1·00	776·8	7·1	7·21	8·2	8·26

The pH was adjusted with the aid of the following buffer solutions: 0·50*M* perchloric acid and 1·00*M* hexamine + 0·50*M* sodium perchlorate for 1·10–2·18 and 4·05–6·45; 0·50*M* perchloric acid and 1·00*M* glycine + 0·50*M* sodium perchlorate for pH 2·28–3·68; 1·00*M* glycine + 0·50*M* sodium perchlorate and 0·50*M* sodium hydroxide, for pH 8·24–10·14; 1·00*M* hexamine + 0·50*M* sodium perchlorate and 0·50*M* sodium hydroxide for pH 10·93–12·95; 10·00*M* sodium hydroxide for pH 12·95–15·80. The solutions with extremely high pH-values were made by the procedure of Schwarzenbach and Sulzberger.[28] Acidity higher than pH 0·00 was expressed as the Hammett function H°. The relation between molarity of perchloric acid and H° was taken from Hammett and Deyrup.[29,30] The ionic strength between pH 1·10 and 12·95 was kept constant (0·10*M*). The buffer compositions given above were preferred to the usual borate buffer because of complex formation of borate with the azo derivatives of chromotropic acid in the pH range 8–10.

Methods

Potentiometric titration. The usual titration of a free acid with *ca.* 0·1*M* sodium hydroxide was performed. In such a case, the mass and charge balances are given generally by the well known equations[31]

$$c_L = [L] \sum_n^N [H]^n \beta_n; \qquad 0 \le n \le N, \beta_0 = 1 \tag{1}$$

$$c_B + [H] - [OH] = [L] \sum_n^N (R - n)[H]^n \beta_n, \tag{2}$$

where

$$\beta_n = [H_nL][H]^{-n}[L]^{-1} = \prod_0^N K_n \tag{3}$$

and

$$K_n = [H_nL][H]^{-1}[H_{n-1}L]^{-1}. \tag{4}$$

Here, c_L and c_B are total concentrations of the ligand L and of the base B, R is the number of hydrogen ions of the electrically neutral complex H_RL. After elimination of [L] from (1) and (2) and a simple transformation, we have

$$\sum_n^N (\bar{n} - R + n)[H]^n \beta_n = 0, \tag{5}$$

where the formation function $\bar{n}$ is given by

$$\bar{n} = \frac{c_B + [H] - [OH]}{c_L}. \tag{6}$$

From the pH-titration of n-basic acid (all K_n values are assumed to be close together) n linear equations (5) may be obtained, from which all constants β_n may be calculated, preferably by means of a computer.

If only three different ligand forms (H_nL, $H_{n+1}L$, $H_{n+2}L$) are present in solution, another very simple method may be used for calculation.[32] Instead of (5), we have

$$\frac{(\bar{n} - R - n)}{[H]^2(\bar{n} - R + n + 2)} + \frac{(\bar{n} - R + n + 1)}{[H](\bar{n} - R + n + 2)} \frac{\beta_{n+1}}{\beta_n} + \frac{\beta_{n+2}}{\beta_n} = 0, \tag{7}$$

where

$$\beta_{n+1}/\beta_n = K_{n+1}, \beta_{n+2}/\beta_n = K_{n+1}K_{n+2}. \tag{8}$$

Equation (7) is a suitable form either for graphic or for numerical calculation of both constants K_{n+1} and K_{n+2}.

If the system involves the complexes H_nL and $H_{n+1}L$ only, one has from (7) and (8)

$$K_{n+1} = -\frac{(\bar{n} - R + n)}{[H](\bar{n} - R + n + 1)}, \tag{9}$$

which is convenient for calculation of K_{n+1}.

By use of (5), (7) and (9), all stability constants can be calculated stepwise. An accurate application of the method requires the use of a minimum ligand concentration of 0·005M, which can be fulfilled with all reagents to be investigated.

Spectrophotometric method. Generally the total absorbance of a solution with hydrogen complexes is given by the equation[33]

$$A = \sum_n^N \varepsilon_n[H_nL]; \qquad 0 \leq n \leq N, \tag{10}$$

or, after the introduction of overall stability constants β_n, by

$$A = [L] \sum_n^N \varepsilon_n[H]^n\beta_n. \tag{11}$$

The combination of (11) with (1) gives

$$\sum_n^N (A - c_L\varepsilon_n)[H]^n\beta_n = 0, \tag{12}$$

where ε_n is the molar absorptivity of the complex H_nL. $(N - n + 1)$ complexes are present in the solution; the absorbance A should be measured at $2(N - n + 1)$ different pH-values, which gives $2(N - n + 1)$ linear equations of the type (12), from which, generally by means of a computer, all molar absorptivities ε_n and all stability constants β_n may be calculated. However the application of this procedure is seldom necessary. A much simpler procedure given below is quite satisfactory.

The sole presence of L or a fully protonated complex H_NL may be assumed in extremely alkaline (pH $\sim$ 16) or extremely acidic ($H^\circ \sim -4$) solution. The molar absorptivity of these ligand forms may be easily calculated by means of the equations

$$\varepsilon_0 = A_0/c_L \text{ or } \varepsilon_N = A_N/c_L, \tag{13}$$

where c_L is the total concentrations of the ligand and A_0 or A_N is the measured absorbance.

Because of stepwise formation of hydrogen complexes, there must exist an acidity range in which only the two complex species L and HL are present. In such a case, instead of (12), we have

$$\bar{\varepsilon} = \frac{\varepsilon_0 + \varepsilon_1\beta_1[H]}{1 + \beta_1[H]} = \frac{A}{c_L} \tag{14}$$

and after a transformation

$$(\varepsilon_0 - \bar{\varepsilon})/[H] = \bar{\varepsilon}\beta_1 - \varepsilon_1\beta_1 \tag{15}$$

which is the form suitable for numerical or graphical calculation of ε_1 and β_1.

If in a range of acidity three ligand forms (L, HL and H_2L) are present in the solution, there will

be instead of (12)

$$\bar{\varepsilon} = \frac{\varepsilon_0 + \varepsilon_1\beta_1[\mathrm{H}] + \varepsilon_2\beta_2[\mathrm{H}]^2}{1 + \beta_1[\mathrm{H}] + \beta_2[\mathrm{H}]^2} = \frac{A}{c_\mathrm{L}} \tag{16}$$

and after a transformation

$$(\varepsilon_0 - \bar{\varepsilon})/[\mathrm{H}]^2 + \beta_1(\varepsilon_1 - \bar{\varepsilon})/[\mathrm{H}] = \bar{\varepsilon}\beta_2 - \varepsilon_2\beta_2. \tag{17}$$

This equation makes possible the numerical or graphical calculation of ε_2 and β_2.

An analogous procedure may be deduced for calculations for systems starting from extremely acidic solution.

If there are ranges of acidity in which there is always present just one ligand form (L or HL or H_2L, . . . *etc*) another common and simple method[4] may be used for calculation of K_n. The absorbance is then given by

$$A_n = \varepsilon_n c_\mathrm{L} = \text{const.}; \qquad 0 \leq n \leq N. \tag{18}$$

The stability constant

$$K_n = [\mathrm{H}_n\mathrm{L}][\mathrm{H}]^{-1}[\mathrm{H}_{n-1}\mathrm{L}]^{-1} \tag{19}$$

may be calculated from the condition $[\mathrm{H}_n\mathrm{L}] = [\mathrm{H}_{n-1}\mathrm{L}]$, for which

$$K_n = [\mathrm{H}]_{1/2}^{-1}. \tag{20}$$

The acidity $[\mathrm{H}]_{1/2}$ corresponds to the absorbance

$$A_{1/2} = (\varepsilon_n + \varepsilon_{n-1})c_\mathrm{L}/2 = (A_n + A_{n-1})/2 \tag{21}$$

The appearance of isosbestic points in a set of spectral curves for different acidities proves the possibility of application of this method.[34]

Only the complexes H_nL and $H_{n-1}L$ should be present in solution in the acidity range in which both absorbances A_n and A_{n-1} obey equation(18). Thede pendence of absorbance A on acidity [H] is then expressed by the equation

$$A = \frac{K_n[\mathrm{H}](A_n - A_{n-1})}{1 + K_n[\mathrm{H}]} + A_{n-1} \tag{22}$$

The slope of the A–(pH) curve is then

$$\frac{\mathrm{d}A}{\mathrm{dpH}} = -2{\cdot}303\,\frac{(A_n - A_{n-1})[\mathrm{H}]}{(1 + K_n[\mathrm{H}])^2}\,. \tag{23}$$

For (20), equation (21) is obtained from (22). Equation (23) gives

$$\frac{\mathrm{d}A_{1/2}}{\mathrm{dpH}_{1/2}} = -0{\cdot}575(A_n - A_{n-1}). \tag{24}$$

If only complexes H_nL and $H_{n-a}L$ are formed in the acidity range in which absorbances A_n and A_{n-a} obey equation (18), equation (22) will have the form

$$A = \frac{K_n'[\mathrm{H}]^a(A_n - A_{n-a})}{1 + K_n'[\mathrm{H}]} + A_{n-a} \tag{25}$$

where

$$K_n' = [\mathrm{H}_n\mathrm{L}][\mathrm{H}]^{-a}[\mathrm{H}_{n-a}\mathrm{L}]^{-1}. \tag{26}$$

Instead of (24) we have

$$\frac{\mathrm{d}A_{1/2}}{\mathrm{dpH}_{1/2}} = -0{\cdot}575a(A_n - A_{n-a}). \tag{27}$$

The shape of several A–(pH) curves may be seen from Fig. 1. The equations (25) and (27) are important for a correct understanding of any shape of the A–(pH) curve and also for a correct choice of a suitable calculation method for stability constant determination.

RESULTS

The pH-titration curves of several reagents are given in Fig. 2. A set of typical absorption spectra is shown in Fig. 3. Dependence of molar absorptivity ε for a characteristic wavelength λ on acidity (pH or H°) is given in Figs. 4*A* and *B*. Calculated stability constants compared with previously determined values are collected in

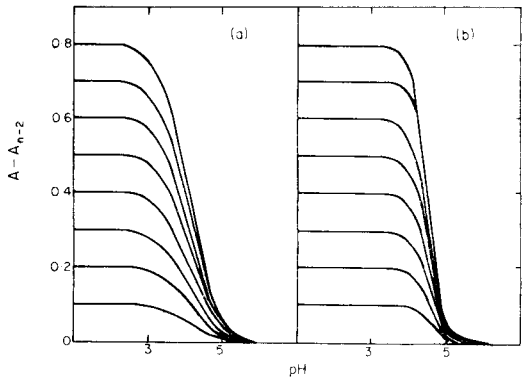


FIG. 1.—Dependence of absorbance for different $A_n - A_{n-a}$ (0·1–0·8) on pH, if two complexes H_nL and $H_{n-a}L$ are present in solution (log $K_n = 4{\cdot}00$). (a); $a = 1$, (b); $a = 2$.

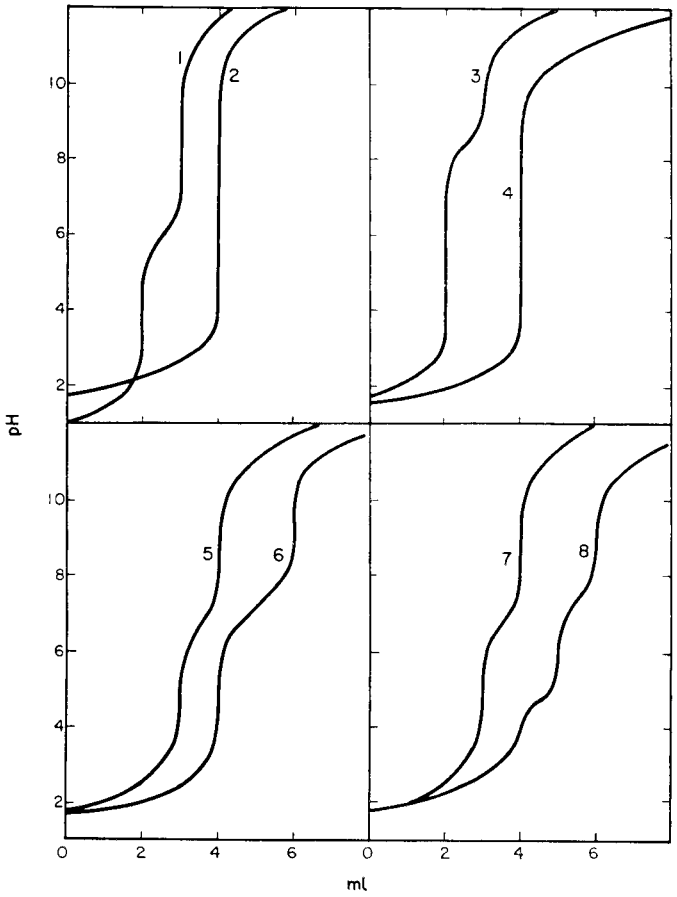


FIG. 2.—pH-Titration curves
1—CA; *2*—DMSA III; *3*—A III *4*—SA III; *5*—CPA I; *6*—CPA III; *7*—AA I; *8*—AA III. 25·00 ml of $4{\cdot}00 \times 10^{-3}M$ reagent solution and of $0{\cdot}096M$ potassium chloride titrated with $0{\cdot}100M$ sodium hydroxide at 20 ± 1°. 20·00 ml of $0{\cdot}050M$ CA were used in the case of curve 1; the apparent added volume of $0{\cdot}100M$ sodium hydroxide should be multiplied by 10.

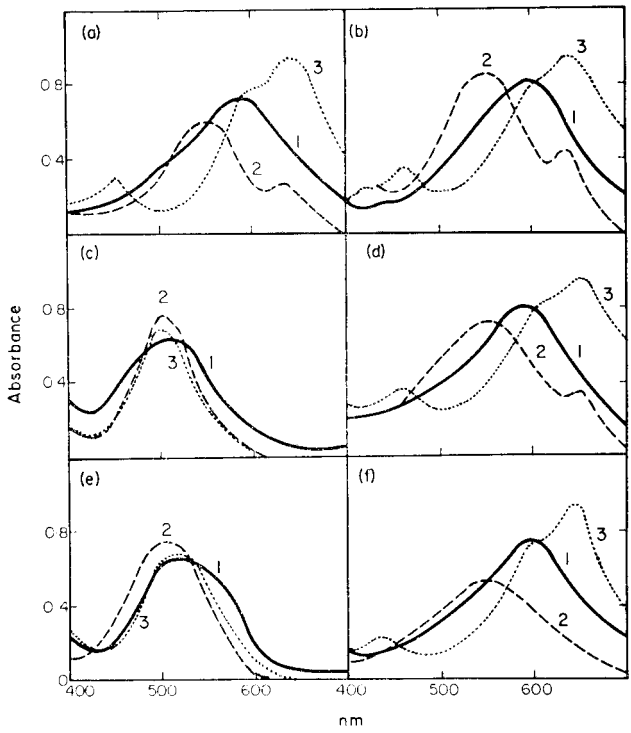


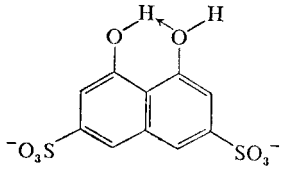
FIG. 3.—Absorption spectra of reagents:
A—A III, *2*—pH 2·46; *B*—DMSA III, *2*—pH 5·80; *C*—CPA I, *2*—pH 5·80; *D*—CPA III, *2*—pH 6·45; *E*—AA I, 2—pH 6·45; *F*—AA III, *2*—pH 5·51. *A*—*f*, *1*—pH 15·80; *3*—H° −3·42; $c_L = 1{\cdot}85 \times 10^{-5} M$ throughout.

Table II. Table III shows wavelengths of isosbestic points and corresponding pairs of complexes which are present in solution. Table IV shows maximum molar absorptivities of individual complex species.

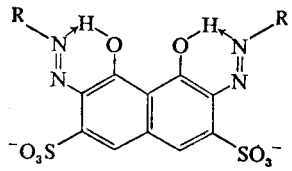
DISCUSSION

The most difficult problem, particularly with bisarylazo derivatives, is the determination of stability constants corresponding to sulpho, phosphono and arsono groups. This problem cannot be solved without the use of a suitable calculation technique and the combination of potentiometric and spectrophotometric experimental methods.

The values found, see Table II, show several interesting relationships between structure and acidity of individual functional groups. For example, the value of log K_1 decreases and the value of log K_2 increases on introduction of arylazo substituents into positions 3 and 6. While structure A is usually assumed for chromotropic acid



A

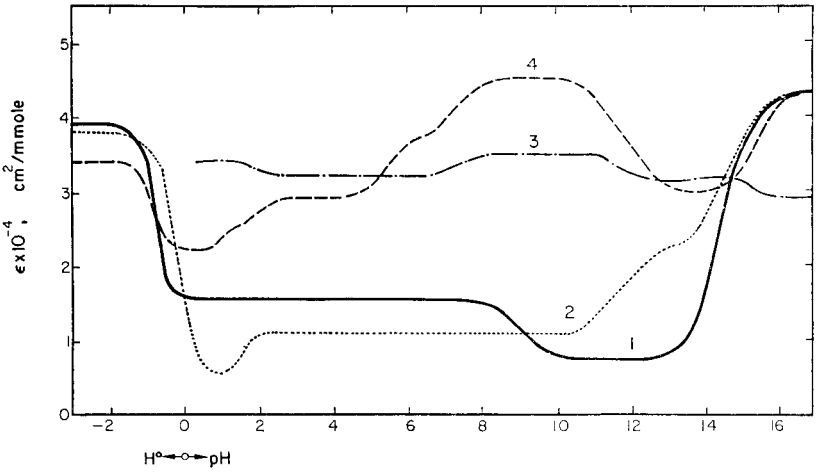


B

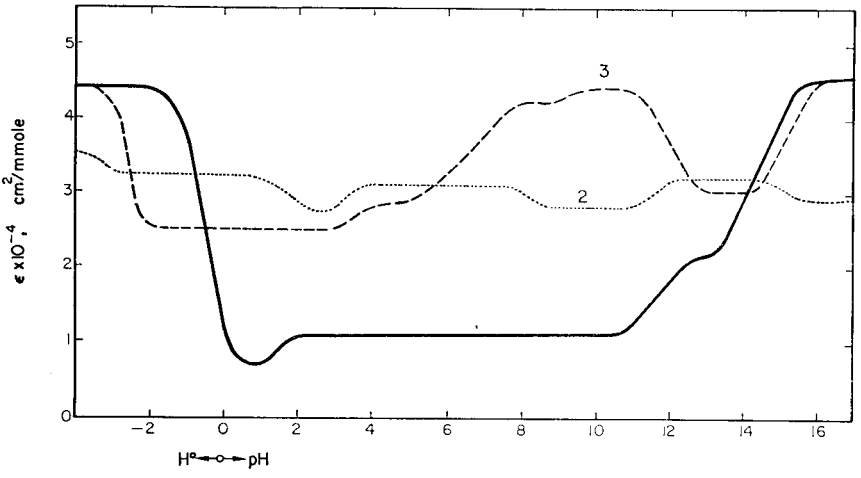
and made responsible for the low value of log K_2, another structure B is more probable for 3,6-bis(arylazo) derivatives. The difference in log K_1 and log K_2 is then mainly due to the field effect. Both log K_1 and particularly log K_2 increase with acid strength and multivalency of acidic groups attached to benzene ring. Thus the following series may be given for this effect:

$$-PO_3^{2-} > -AsO_3^{2-} > -SO_3^- \gg -H$$

The difference between phosphono and arsono groups on the one hand and sulpho groups on the other is in the chromogenic behaviour of reagents in strongly alkaline



(a)



(b)

FIG. 4.—Dependence of molar absorptivity ε on pH.
A: *1*—A III; *2*—SA III; *3*—CPA I; *4*—CPA III. *B*: *1*—DMSA III; *2*—AA I; *3*—AA III. $c_L = 4{\cdot}00 \times 10^{-4} M$ and wavelength 600 nm throughout, except for *A3* and *B2*, where the wavelength was 520 nm.

TABLE II.—STEPWISE STABILITY CONSTANTS OF HYDROGEN COMPLEXES

Reagent	$\log K_n = \log [H_nL][H]^{-1}[H_{n-1}L]^{-1}$									
	$n = 1$	2	3	4	5	6	7	8	9	10
CA	15·5±0·1**	5·45±0·03*	0·73±0·03*	0·61±0·02*						
	15·6#	5·36#								
A I	14·46#	9·29#								
A III	14·5±0·1**	8·76±0·04*	0·81±0·07*	0·58±0·05*	−1·1±0·1**	−1·1±0·1**				
		8·9±0·1**								
SA III	14·4±0·1**	11·61±0·20**	2·8±0·2*	2·4±0·2*	0·8±0·1*	0·6±0·1*	−0·3±0·1**	−0·3±0·1**		
	14·5##	11·7##	2·9##	2·3##	1·9##	0·9##	0·3##	−2·0##		
DMSA III	14·4±0·1**	11·77±0·17**	2·9±0·2*	2·5±0·2*	0·8±0·1*	0·6±0·1*	−0·5±0·1**	−0·5±0·1**		
	14·7***	11·8***	5·7***	4·5***	3·4***	2·4***	2·0***	−1·5***		
CPA I	15·2±0·1**	11·75±0·13**	7·28±0·15**	1·85±0·21*	0·8±0·1*	0·6±0·1*				
CPA III	15·3±0·1**	12·15±0·11**	7·20±0·15**	5·47±0·25**	2·5±0·1*	1·5±0·1*	0·8±0·1*	0·6±0·1*	−1·1±0·1**	−1·1±0·1**
	14·6###	11·1###	9·4###	7·0###	4·2###	1·5###	0·6###	0·3###	−0·5###	−2·1###
AA I	15·0±0·1**	11·62±0·05**	8·20±0·11**	3·52±0·11*	0·8±0·1*	0·6±0·1*				
	12·54‡	9·31‡	7·52‡	3·50‡	1·30‡	0·00‡				
AA III	15·1±0·1**	11·98±0·11**	9·05±0·20**	6·27±0·20**	3·4±0·1*	1·6±0·1*	0·8±0·1*	0·6±0·1*	−2·7±0·1**	−2·7±0·1**
	12·33¶	7·48¶	5·25¶	5·25¶	2·41¶	2·41¶	0·00¶	−2·55¶		
	14·6¶¶	11·7¶¶	9·0¶¶	7·1¶¶	5·2¶¶	3·8¶¶	−0·6¶¶	−1·3¶¶	−2·4¶¶	−5·5¶¶
	11·85‡‡	9·30‡	7·64‡	6·77‡‡	5·05‡	3·64‡	2·96‡	1·89‡		

\# Determined by Schwarzenbach and co-workers.[26,27] ## See ref. 8. ### See ref. 13.
* pH-method. ** Spectrophotometry. *** See ref. 14.
‡ See ref. 15. ¶ See ref. 3. ¶¶ See ref. 7.
‡‡ See ref 20.

TABLE III.—ISOSBESTIC POINTS IN ABSORPTION SPECTRA OF REAGENTS

Reagent	Complexes	ΔpH	$\lambda_{isos}nm$
A III	L, HL	15·0–13·0	410, 530
	HL, H_2L	10·0–7·0	420, 495
DMSA III	L, HL	15·0–13·8	370, 415, 515, 685
	HL, H_2L	13·5–12·0	380, 480, 520, 590
	H_2L, H_3L	10·0–8·0	330, 430, 490, 690
CPA I	L, HL	15·4–14·0	395, 430, 480, 545
	HL, H_2L	12·0–10·5	395, 440, 520
	H_2L, H_3L	8·0–6·0	410, 470
CPA III	L, HL	15·5–14·0	415, 510, 680
	HL, H_2L	13·0–11.5	370, 530, 620
	H_2L, H_3L	8·0–6·5	470, 570, 690
AA I	L, HL	15·4–13·5	390, 460, 495, 540
	HL, H_2L	12·2–10·9	380, 430, 490
	H_2L, H_3L	9·0–7·0	400, 460, 530
AA III	L, HL	15·5–14·0	370, 415, 480
	HL, H_2L	13·0–11·0	320, 450, 690
	H_2L, H_3L	9·0–7·5	330, 410, 500
	H_3L, H_4L	6·5–5·0	450, 580

TABLE IV.—MAXIMUM MOLAR ABSORPTIVITIES OF INDIVIDUAL HYDROGEN COMPLEXES

Reagent	$\varepsilon \times 10^{-4}$, $cm^2/mmole$ for wavelength λ, nm					
	L	HL	H_2L	H_3L	H_4L	H_NL*
A III	4·35	2·10	3·50	—	5·50	
	(660)	(540)	(550)		(650)	
SA III	4·41	2·93	4·19	—	3·98	5·10
	(610)	(580)	(580)		(580)	(670)
DMSA III	4·40	2·94	4·20	—	4·00	5·10
	(610)	(580)	(580)		(580)	(670)
CPA I	3·46	3·76	3·87	3·50		
	(525)	(530)	(520)	(500)		
CPA III	4·37	3·58	4·50	3·95		5·10
	(600)	(580)	(600)	(570)		(670)
AA I	3·45	3·81	3·70	3·85		
	(520)	(530)	(505)	(500)		
AA III	4·52	3·18	3·98	3·78	4·05	5·10
	(600)	(585)	(600)	(560)	(535)	(670)

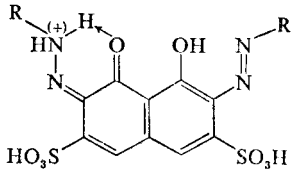
* N is the maximum number of hydrogen ions bounded; for SA III and DMSA III, $N = 8$; for CPA III and AA III, $N = 10$.

solution; see Figs. 4*A* and *B*. The bathochromic effect of arsono and phosphono groups is stronger than that of the sulpho groups.

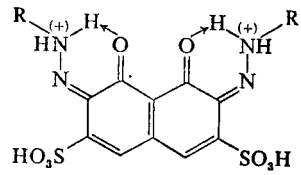
The acidities of sulpho, phosphono and arsono groups (attached to a benzene ring) are relatively weakly affected by the field effect. The colour shifts connected with their dissociation are also smaller, which causes difficulties in the spectrophotometric determination of stability constants.[35] Both sulphonic groups of chromotropic acid have almost the same acidity for all derivatives. The dissociation is connected with a very small colour effect so that the spectrophotometric determination of stability constants is almost impossible. Contrary to Palei and co-workers,[20] who prefer the spectrophotometric method, the pH method appears the more important in this case. According to the same authors,[20] the main break in a potentiometric titration of

Arsenazo III is connected with dissociation of three protons; however, the curves in Fig. 2 show that all reagents, Arsenazo III, Chlorophosphonazo III, Sulphonazo III and Dimethylsulphonazo III, give a break connected with dissociation of four protons, which is in good agreement with the symmetric structure of all reagents and with the known acidities of arsono, phosphono and sulpho groups.

The hydrogen complex formation in extremely acidic solution (positively charged complexes) gives rise to a very significant colour shift from wine-red to grass-green. The shape of absorbance–pH curves, see Figs. 1 and 4*A*, 4*B*, shows that association or dissociation of two protons occurs in contrast to the results of molecular orbital calculations by Savvin and Kuzin,[24] who assume the reaction with one proton only. The structures **C** and **D** appear as most probable.



C



D

There is poor agreement among values of H° determined by different authors for the same acid.[36] Several differences in published values of stability constants are also caused by these circumstances. Perchloric acid is well characterized from this point of view;[29,30] unfortunately the preparation of reagent solutions in concentrated perchloric acid should be performed at low temperatures only (about −5°) because of the oxidation effect of perchloric acid.

Acidic and chromogenic properties of individual reagents establish the complexing and metallochromic behaviour with metal ions. Thus Arsenazo III is the most important for complex formation in extremely acidic solution, where the complexes with uranium(IV), zirconium, hafnium and thorium are formed. The predominant composition of these complexes has the molar ratio of metal:ligand as 1:2. With ions of lower hydrolytic tendencies, such as yttrium, lanthanons, uranium(VI) and palladium(II), the optimum pH is about 2·9, because of the bathochromic shift of reagent alone due to formation of the H_4L complex (log $K_5 = 3{\cdot}4$, see Table II). Usually complexes with a molar ratio of metal:ligand of 1:1 are formed and, in a smaller degree, 1:2. Arsenazo III is less important for the complexation of metal ions at higher pH (such as magnesium, calcium, strontium, barium, *etc*), because of a relatively strong bathochromic effect of reagent and weak stability of the metal complexes.

The acidity range for Chlorophosphonazo III metal complex formation for $H^\circ < 0$ is very small because of the bathochromic shift of reagent alone, which occurs from about $H^\circ = -0{\cdot}5$ (see Fig. 4*A*). For these reasons the most important acidity ranges for metal complex formation are about pH 2·0 [uranium(VI), yttrium and lanthanons] and 7·0 (magnesium, calcium, strontium, barium and radium), in both cases because of a bathochromic effect of reagent alone due to the formation of complexes H_4L (log $K_5 = 2{\cdot}5$) and H_2L (log $K_3 = 7{\cdot}20$). In the first case, the most usual molar ratio of metal to ligand is 1:2, and in the second, 1:1. Reagents of the Sulphonazo III type have two suitable acidity ranges for metal complex formation. The first

is about pH 2·0 [palladium(II), barium], the second about pH 7·0 (calcium, strontium, barium and radium), both because of the bathochromic shift of reagent alone due to the formation of H_4L and H_2L complexes (see Table II). Complexes with a molar ratio of metal to ligand of 1:1 are formed throughout.

Zusammenfassung—Mit potentiometrischen und spektrophotometrischen Methoden wurden die Stabilitätskonstanten der protonierten Formen folgender Säuren ermittelt: 4,5-Dihydroxynaphthalin-2,7 disulfonsäure (Chromotropsäure, CA); 3,6-Bis(phenylazo)-4,5-dihydroxynaphthalin-2,7-disulfonsäure (Azo III, A III); 3,6-Bis(2′-sulfophenylazo)-4,5-dihydroxynaphthalin-2,7-disulfonsäure (Sulfonazo III, SA III); 3,6-Bis (4′-methyl-2-sulfophenylazo)-4,5,dihydroxynaphthalin 2,7-disulfonsäure (Dimethylsulfonazo III, DMSA III); 3-(4′-Chloro-2′-phosphonophenylazo-4,5-dihydroxynaphthalin-2,7-disulfonsäure (Chlorophosphonazo I, CPA I); 3,6-Bis(4′-chloro-2′-phosphonophenylazo)-4,5-dihydroxynaphthalin-2,7-disulfonsäure (Chlorophosphonazo III, CPA III); 3-(2′-Arsonophenylazo)-4,5-dihydroxynaphthalin-2,7-disulfonsäure (Arsenazo I, AA I) und 3,6-Bis(2′-arsonophenylazo)-4,5-dihydroxynaphthalin-2,7-disulfonsäure (Arsenazo III, AA III).

Résumé—On a étudié les méthodes potentiométrique et spectrophotométrique pour la détermination des constantes de stablité des complexes hydrogène de l'acide 4,5-dihydroxynaphtalène 2,7-disulfonique (acide chromotropique, CA), de l'acide 3,6-bis (phénylazo) 4,5-dihydroxynaphtalène 2,7-disulfonique (Azo III, A III), de l'acide 3,6-bis(2′-sulfophénylazo)4,5-dihydroxynaphtalène 2,7-disulfonique (Sulfonazo III, SA III), de l'acide 3,6-bis (4′-methyl 2′-sulfophénylazo) 4,5-dihydroxynaphtalène 2,7-disulfonique (Diméthylsulfonazo III, DMSA III), de l'acide 3-(4′-chloro 2′-phosphonophénylazo) 4,5-dihydroxynaphtalène 2,7-disulfonique (Chlorophosphonazo I, CPA I), de l'acide 3,6-bis (4′-chloro 2′-phosphonophénylazo) 4,5-dihydroxynaphtalène 2,7-disulfonique (Chlorophosphonazo III, CPA III), de l'acide 3-(2′-arsonophénylazo)4,5-dihydroxynapthalene 2,7- disulfonique (Arsénazo I, AA I) et de l'acide 3,6-bis(2′-arsonophénylazo) 4,5-dihydroxynaphtalène 2,7-disulfonique (Arsenazo III, AA III).

REFERENCES

1. S. B. Savvin, *Arsenazo III*, Atomizdat, Moscow 1966.
2. B. Buděšínský, in *Chelates in Analytical Chemistry* (eds. H. A. Flaschka and A. J. Barnard, Jr.), Vol. 2, Dekker, New York, 1969.
3. *Idem, Collection Czech. Chem. Commun.*, 1963, **28,** 2902.
4. *Idem, Z. Anal. Chem.*, 1964, **202,** 96.
5. *Idem, ibid.*, 1964, **206, 401.**
6. *Idem, ibid.*, 1965, **207,** 105.
7. *Idem, ibid.*, 1965, **207,** 247.
8. B. Buděšínský and D. Vrzalová, *ibid.*, 1965, **210,** 161.
9. B. Buděšínský and K. Haas, *ibid.*, 1965, **210,** 263.
10. *Idem, ibid.*, 1965, **214,** 325.
11. B. Buděšínský and S. B. Savvin, *ibid.*, 1965, **214,** 189.
12. A. Bezděková and B. Buděšínský, *Collection Czech. Chem. Commun.*, 1966, **31,** 199.
13. B. Buděšínský, K. Haas and A. Bézděková, *ibid.*, 1967, **32,** 1528.
14. B. Buděšínský, D. Vrzalová and A. Bézděková, *Acta Chim. Acad. Sci. Hung.*, 1967, **37, 52.**
15. A. F. Kuteinikov, *Zavodsk. Lab.*, 1962, **28,** 1179.
16. S. B. Savvin, *Zh. Analit. Khim.*, 1962, **17,** 785.
17. *Idem, Talanta*, 1961, **8,** 673.
18. A. A. Nemodruk, *Zh. Analit. Khim.*, 1964, **19,** 790.
19. I. P. Alimarin, S. B. Savvin and L. A. Okhanova, *Talanta*, 1968, **15,** 601.
20. P. N. Palei, N. I. Udaltsova and A. A. Nemodruk, *Zh. Analit. Khim.*, 1967, **22,** 1797.
21. H. Khalifa, M. A. Khater and A. A. El-Siraly, *Z. Anal. Chem.*, 1968, **237,** 111.

22. L. A. Gribov, E. L. Kuzin and S. B. Savvin, *Zh. Analit. Khim.*, 1967, **22,** 1790.
23. *Idem, ibid.*, 1968, **23,** 5.
24. S. B. Savvin and E. L. Kuzin, *Talanta*, 1968, **15,** 913.
25. B. Buděšínský, *ibid.*, 1968, **15,** 1063.
26. J. Heller and G. Schwarzenbach, *Helv. Chim. Acta*, 1951, **34,** 1876.
27. G. Schwarzenbach and G. Anderegg, in *Stability Constants* (eds. L. G. Sillén and A. E. Martell), p. 712. Chemical Society, London, 1964.
28. G. Schwarzenbach and R. Sulzberger, *Helv. Chim. Acta*, 1944, **27,** 348.
29. L. P. Hammett and A. J. Deyrup, *J. Am. Chem. Soc.*, 1932, **54,** 2721.
30. T. G. Bonner and J. C. Lockhart, *J. Chem. Soc.*, 1957, 2840.
31. B. P. Block and G. H. McIntyre, *J. Am. Chem. Soc.*, 1953, **75,** 5667.
32. G. Schwarzenbach, A. Willi and R. O. Bach, *Helv. Chim. Acta*, 1947, **30,** 1303.
33. M. Murakami, T. Yoshino and S. Harasawa, *Talanta*, 1967, **14,** 1293.
34. B. Buděšínský and K. Haas, *Collection Czech. Chem. Commun.*, 1964, **29,** 1006.
35. Z. Slovák, J. Fischer and J. Borák, *Talanta*, 1968, **15,** 831.
36. M. A. Paul and F. A. Long, *Chem. Revs.*, 1957, **51,** 1.

Talanta, 1969, Vol. 16, pp. 1289 to 1296. Pergamon Press. Printed in Northern Ireland

DIFFERENTIAL REFLECTANCE SPECTROPHOTOMETRY—I

HIGH-REFLECTANCE METHOD FOR DETERMINATION OF MICRO AMOUNTS OF SUBSTANCES RESOLVED ON THIN PLATES

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Summary—The basic principles of differential high-reflectance spectroscopy are discussed from the standpoint of the determination of substances resolved on chromatoplates. Results obtained with the use of two systems, nickel dimethylglyoximate or copper neocuproinate adsorbed on cellulose, are used as illustrations. A graphical method for selecting the optimum concentration range for analysis and for determining the maximum accuracy attainable is also outlined. When contrasted with the conventional method of measuring reflectance, the technique promises substantially increased accuracy over a wider concentration range and seems particularly suited to the analysis of trace amounts of material.

SPECTRAL reflectance has found application in the solution of a variety of problems in chemical analysis, particularly those relating to paper and thin-layer chromatography.[1–7] A critical study of the optimum concentration range for reflectance spectrophotometric analysis has revealed that, as in absorption spectroscopy, precision is lowest for very high or very low reflectance.[8] In absorption spectroscopy this limitation may often be circumvented by use of a differential method.[9–11] This study was undertaken to ascertain whether a similar approach could be employed in reflectance spectrophotometry.

EXPERIMENTAL

Reagents

Reagents were of analytical grade or comparable purity. Dilution series of the various cations were prepared from aqueous stock solutions containing 3·177 g of $Cu(NO_3)_2 \cdot 3H_2O$ per litre and 2·937 g of $Ni(NO_3)_2 \cdot 6H_2O$ per litre.

The stock solutions were applied as spots by means of a 10-μl Hamilton microsyringe to 200 × 50 × 3·5 mm plates coated with a 17:3 mixture of water and MN-cellulose powder 300 (Macherey, Nagel and Co.; 516 Düren, Germany), applied with a Desaga-Brinkman Model SII applicator with gate set at 0·50 mm. After the plates has been dried, traces of iron found to be present were displaced to the top of the plate by ascending development with 12*M* hydrochloric acid–1-butanol (1:3). These pretreated plates were then dried, spotted with the test solution, air-dried for 5 min, developed with the hydrochloric acid–butanol mixture, dried for 30 min at 75°, sprayed with detecting reagent, and again dried for 20 min at 75°. Spray reagents were 29% ammonia–1% ethanolic dimethylglyoxime solution (1:9) for nickel, and 20% hydroxylamine hydrochloride solution–saturated ethanolic neocuproine solution (1:2) for copper.

Apparatus

Reflectance measurements were made with a Beckman Model DU Spectrophotometer fitted with a standard reflectance attachment (Catalogue No. 2580). When used for conventional reflectance

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measurement the instrument was set to read 0 with the photocell in darkness, and 100 when the photocell was exposed to the light reflected from the reference standard (the cellulose of the plate measured). For differential reflectance measurements the instrument was set to read 0 when the photocell was exposed to light reflected from a reference sample somewhat more concentrated than the sample being analysed.

RESULTS AND DISCUSSION

As in absorption spectroscopy, it is assumed that the instrument reading is a linear function of the light reflected from the sample. The basic equation relating reflectance R to light power measured, is

$$R = I_X/I_0 \tag{1}$$

where I_X and I_0 are the light intensities reflected by sample and non-absorbing reference standard respectively. In the differential method, where zero reflectance is set to correspond to that of a standard rather more concentrated than the test sample, $R_0 = I_s/I_0$, so that in effect the scale is expanded (*cf.* Reilley and Crawford[11]) and the differential reflectance, R_d is given by

$$R_d = (I_X - I_s)(I_0 - I_s) \tag{2}$$

The most general theory treating diffuse reflection and the transmission of light-scattering layers was developed by Kubelka and Munk.[12,13] When the sample is diluted with a non- or low-absorbing powder and its reflected light is measured relative to the light reflected by the pure diluent powder, the equation derived by Kubelka and Munk[12] can be written in the form:

$$c = k'(1 - R_{X0})^2/2R_{X0} \tag{3}$$

where c is the molar concentration, R_{X0} is the reflectance of the sample relative to a non- or low-absorbing standard, and k' is a constant. This equation is the one usually used in conventional reflectance spectrophotometry.

A similar expression for differential spectrophotometry can be obtained from equation (2) by converting light intensities into the corresponding reflectances. Division by I_0 gives

$$R_d = (R_{X0} - R_{s0})(1 - R_{s0}) \tag{4}$$

where R_{s0} is the reflectance of the differential standard. Rearranging and combining equations (3) and (4) gives

$$c = k'\{(1 - R_{s0})(1 - R_d)\}^2/\{2R_d(1 - R_{s0}) + R_{s0}\}. \tag{5}$$

If the concentration of the differential standard is so great that the incident light is completely absorbed, equation (5) reduces to equation (3).

Equation (5) indicates that slopes of plots obtained for systems conforming to the Kubelka-Munk equation should be independent of the concentration of the differential standard. To illustrate this, the nickel–dimethylglyoxime and copper–neocuproine complexes, both adsorbed on cellulose, were taken as models. The results with varying concentrations of the complexes used as differential standards are shown in Figs. 1 and 2. When these results are graphed in the form $\{(1 - R_{s0})(1 - R_d)\}^2/\{R_d(1 - R_{s0}) + R_{s0}\}$ *vs.* concentration of the complex, the plots in Figs. 3 and 4 result. Results obtained when the zero adjustment was made with the photocell in darkness are included for comparative purposes and correspond to those obtained

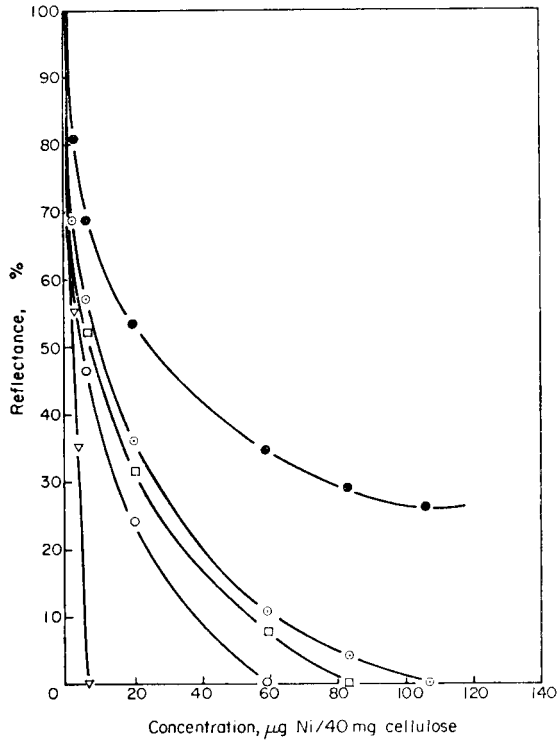

FIG. 1. Reflectance at 540 nm, of nickel dimethylglyoximate adsorbed on cellulose, as a function of concentration.
Differential standard concentrations (μg Ni/40 mg cellulose):
—●— photocell in darkness; —○— 107; —□— 83; —⊙— 59; —▽— 5·9.

in conventional reflectance spectrophotometry. These results demonstrate the close conformity of the nickel–dimethylglyoxime–cellulose system to the Kubelka-Munk equation, and so it is not surprising that the plots of the data obtained by means of the high-reflectance method take the form of straight lines which can be superimposed. By contrast, the departure from linearity observed with the copper–neocuproine–cellulose system, whether the results were obtained with the use of the conventional or the high-reflectance technique, indicates a less than ideal behaviour with respect to the Kubelka-Munk equation.

Regardless of whether a system conforms to the Kubelka-Munk equation or not, the optimum concentration range for the high-reflectance spectrophotometric method can be deduced by computing the relative error, dc/c, caused by reading error. Taking the derivative of equation (5) with respect to R_d, the relative error in c is:

$$\frac{dc}{c} = \frac{k'(1 - R_{s0})^2(1 - R_d)(R_{s0}R_d - R_d - R_{s0} - 1)dR_d}{2c\{R_d(1 - R_{s0}) + R_{s0}\}^2} \qquad (6)$$

If the reading error is assumed to amount to one reflectance unit, *i.e.*, $dR_d = 0{\cdot}01$, it is a simple matter to compute the relative error (in %) in the calculated concentration. The inverse of the slope at concentration c, *viz.* $k'/2$, may be obtained from Kubelka-Munk plots similar to Figs. 3 and 4, while R_{s0} may be obtained by determining the

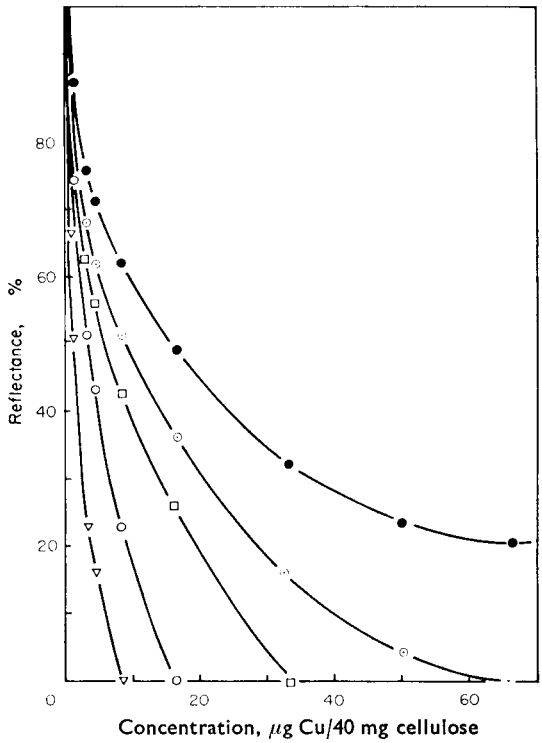

FIG. 2.—Reflectance at 448 nm, of copper neocuproinate adsorbed on cellulose, as a function of concentration.
Differential standard concentrations (μg Cu/40 mg cellulose):
—●— photocell in darkness; —⊙— 67; —□— 33; —○— 17; —▽— 8·4.

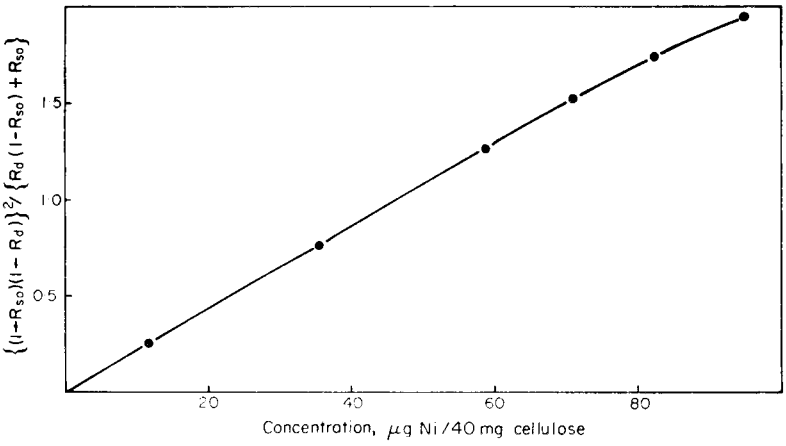

FIG. 3.—$\{(1 - R_{so})(1 - R_s)\}^2/\{R_d(1 - R_{so}) + R_{so}\}$ *vs.* concentration of the nickel–dimethylglyoxime complex. Each point represents the mean value obtained for a given concentration measured relative to a series of differential standards having different concentrations. The range is indicated by the size of the spots.

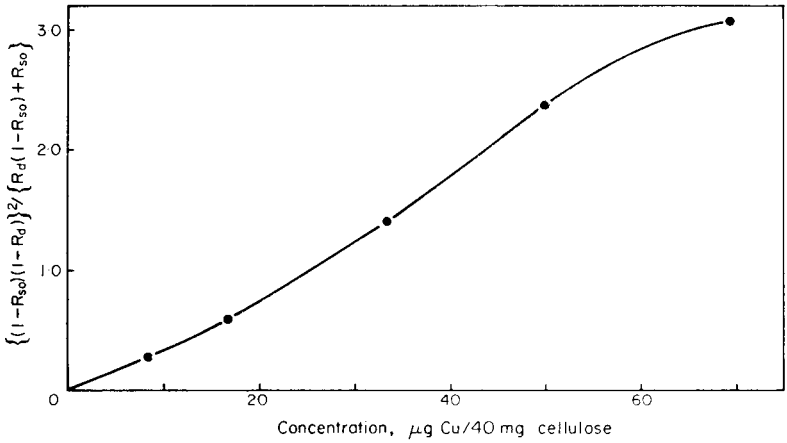

FIG. 4.—$\{(1 - R_{so})(1 - R_d)\}^2/\{R_d(1 - R_{so}) + R_{so}\}$ *vs.* concentration of the copper–neocuproine complex. Each point represents the mean value obtained for a given concentration measured relative to a series of differential standards having different concentrations. The range is indicated by the size of the spots except for the 50 μg point, where it was $\pm 0{\cdot}09$.

reflectance of the differential standard conventionally. When this was done over a range of concentrations for nickel dimethylglyoximate adsorbed on cellulose and for copper neocuproinate adsorbed on cellulose, the plots shown in Figs. 5 and 6 resulted. Similar families of curves were obtained when the error in the concentration was estimated by a graphical method described elsewhere.[8]

The optimal reflectance and concentration ranges for analyses involving the two systems under consideration, as well as the analysis error to be expected as a result of an error amounting to 1% R, are indicated in Figs. 5 and 6 and summarized in

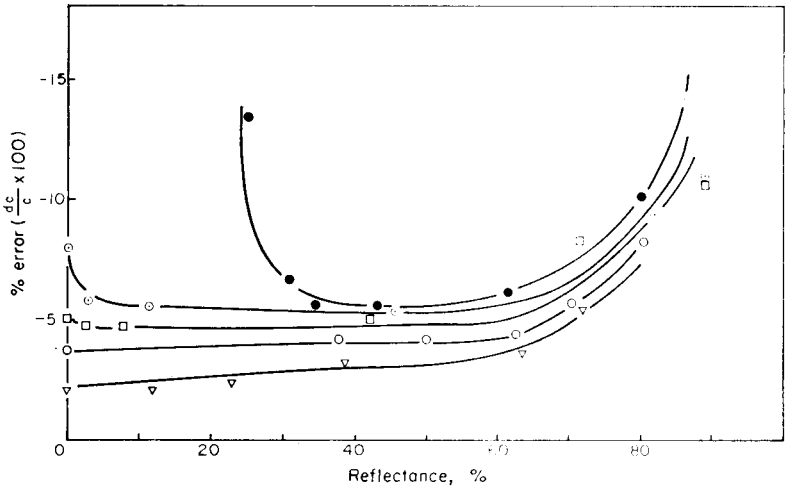

FIG. 5.—Relative error arising from an error of 1% R, computed with the use of equation (6), as a function of reflectance for nickel dimethylglyoximate adsorbed on cellulose.
Concentration of differential standard, μg Ni/40 mg cellulose:
—●— photocell in darkness; —○— 107; —□— 83; —○— 59; —▽— 5·9.

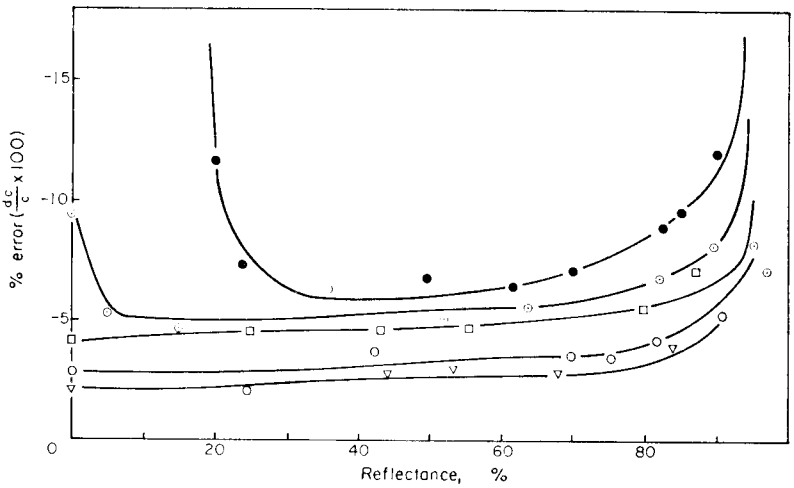

FIG. 6.—Relative error arising from an error of 1% R, computed with the use of equation (6), as a function of reflectance for copper neocuproinate adsorbed on cellulose.
Concentration of differential standard, μg Cu/40 mg cellulose:
—●— photocell in darkness; —○— 67; —□— 33; —○— 17; —▽— 8·4.

Table I, and show well some of the advantages afforded by the high reflectance method as contrasted with the conventional procedure for measuring the reflectance of a test sample. For example, the optimum reflectance and concentration ranges for analysis are both extended to lower values with the use of the high reflectance method, this extension increasing as the concentration of the differential standard decreases, and approaching a value of 0% R when the concentration of the standard falls in the range 2·1–2·7 μg/mg of cellulose for nickel and 0·8–1·7 μg/mg of cellulose for copper. At the same time, a decrease in the concentration of the differential standard is accompanied by an extension of the optimum concentration range to lower concentrations. In the case of nickel, this limit is lowered from the value 0·12 μg/mg of cellulose, obtained by means of conventional reflectance spectroscopy, to 0·025 μg/mg when

TABLE I.—OPTIMUM REFLECTANCE AND CONCENTRATION RANGES FOR ANALYSIS

System	Concentration of differential standard, μg/40 mg of cellulose	Optimum range: Reflectance, %R	Optimum range: Concentration, μg/mg of cellulose	% Error per 1% R: lower limit	% Error per 1% R: mid-range	% Error per 1% R: upper limit
Nickel–dimethylglyoxime	5·9	0–70	1·0–5·9	−2		−5
	59	0–70	1·3–59	−3		−6
	71	0–70	1·5–71	−4		−6
	83	0–70	2·3–83	−5	−4	−6
	107	5–70	3·0–76	−6	−5	−7
	Photocell in darkness	30–70	5·0–76	−6		−7
Copper–neocuproine	8·4	0–80	0·5–8·4	−2		−3
	17	0–80	0·8–17	−3		−4
	33	0–80	1·2–33	−4		−5
	67	10–80	1·5–40	−5		−7
	Photocell in darkness	30–80	2·0–36	−7	−6	−8

a 0·15 μg/mg differential standard is used. Similarly reduction by a factor of four is obtained for copper by using a 0·21 μg/mg differential standard.

Of equal import is the fact that at relatively high concentrations within the optimum concentration range the relative error in the analysis arising from an error of 1% R decreases with a decrease in the concentration of the differential standard. It is thus possible, by a judicious choice of the differential standard, to increase the accuracy of the analysis appreciably. For example, the error in the analysis by conventional reflectance spectroscopy of a sample consisting of 5·0 μg of nickel adsorbed on 40 mg of cellulose is ~—7%. This can be reduced to ~—2% by the simple expedient of using 0·15 μg Ni/mg of cellulose as the differential standard. In the same way, error in the determination of 7·0 μg of copper adsorbed on 40 mg of cellulose can be reduced by a factor of 3·5 by employing a 0·21 μg/mg differential standard. While the accuracy of the analysis of copper neocuproinate samples of relatively low concentration falling within the optimum range was similarly increased by the expedient of reducing the concentration of the differential standard, this effect was less pronounced in the case of the nickel–dimethylglyoxime system. It therefore seems that maximum accuracy would be attained in either case by working in the upper portion of the optimum concentration range, with a differential standard of higher concentration than the test sample.

It has been assumed throughout that dR_d is independent of R_d and of the differential standard employed. The validity of this assumption was tested experimentally by preparing a concentration series of nickel–dimethylglyoxime–cellulose samples with reflectance extended over the entire reflectance scale, and then determining the reflectance of each of its members, in turn, relative to differential standards containing 10, 25, 35 and 70 μg of nickel. Several measurements were made for each combination of test and standard samples. The standard derivation obtained for every set of replicate measurements fell randomly in the range 0·1–0·3% R.

CONCLUSIONS

It would seem that the application of the high-reflectance technique makes it possible to analyse samples with an error as low as 2%, and in no case with an error greater than 7%, for a reading error of 1% R. This represents a substantial increase in accuracy over that afforded by the conventional method of measuring reflectance, for which the minimum error is ~7% under optimal conditions.[8] Since the precision generally encountered in reflectance analysis is of the order of 0·3% R, an overall error ranging from 0·6% to 2% could reasonably be expected when the technique described here is used. In practice it is usually unnecessary to prepare an error curve to determine the best standard to use; to increase the accuracy of the analysis it suffices to use a standard with a concentration somewhat greater than that of the test sample.

For determination of substances resolved on thin plates, the high-reflectance technique is especially useful for systems where the absorption is low that conventional reflectance spectroscopy cannot be used, and will often give results accurate enough for preconcentration not to be needed.

Acknowledgement—The authors wish to acknowledge the support provided by the National Institutes of Health, Grant GM-12925.

Zusammenfassung—Die Grundprinzipien der Differenzspektroskopie gut reflektierender Oberflächen werden vom Standpunkt der Bestimmung auf chromatographischen Platten getrennter Stoffe diskutiert. Zur Illustration werden Ergebnisse verwendet, die an zwei Systemen gewonnen wurden: an Cellulose adsorbiertem Dimethylglyoximnickel oder Neocuproinkupfer. Außerdem wird eine graphische Methode angegeben, um den für die Analyse optimalen Konzentrationsbereich und die erreichbare maximale Genauigkeit zu finden. Verglichen mit der üblichen Methode der Reflexionsmessung verspricht das Verfahren erheblich größere Genauigkeit in einem größeren Konzentrationsbereich und scheint besonders geeignet zur Analyse von Spurenmengen.

Résumé—On discute des principes de base de la spectroscopie à fort pouvoir réflecteur différentielle du point de veu de la détermination de substances séparées sur chromatoplaques. On utilise, en tant qu'exemples, les résultats obtenus avec l'emploi de deux systèmes, le diméthylglyoximate de nickel ou le néocuproinate de cuivre adsorbés sur cellulose. On donne aussi un aperçu d'une méthode graphique pour sélectionner le domaine de concentration optimal pour l'analyse et pour déterminer la précision maximale que l'on peut atteindre. Lorsqu'on l'oppose à la méthode ordinaire de mesure du pouvoir réflecteur, la technique offre une précision nettement accrue dans un plus large domaine de concentrations et semble particulièrement convenable à l'analyse de produits à l'état de traces.

REFERENCES

1. K. Yamaguchi, S. Fujii, T. Tabata and S. Kato, *J. Pharm. Soc. Japan*, 1954, **74,** 1322.
2. K. Yamaguchi, S. Fukushima and M. Ito, *ibid.*, 1955, **75,** 556.
3. M. M. Frodyma, R. W. Frei and D. J. Williams, *J. Chromatog.*, 1964, **13,** 61.
4. M. M. Frodyma and R. W. Frei, *ibid.*, 1964, **15,** 501.
5. *Idem, ibid.*, 1965, **17,** 131.
6. R. M. McCready and E. A. McComb, *Anal. Chem.*, 1954, **26,** 1645.
7. M. M. Frodyma, V. T. Lieu and R. W. Frei, *J. Chromatog.*, 1965, **18,** 520.
8. V. T. Lieu and M. M. Frodyma, *Talanta*, 1966, **13,** 1319.
9. R. Bastian, *Anal. Chem.*, 1949, **21,** 972.
10. C. F. Hiskey, *ibid.*, 1949, **21,** 1440.
11. C. N. Reilley and C. M. Crawford, *ibid.*, 1955, **27,** 716.
12. P. Kubelka and F. Munk, *Z. Techn. Physik*, 1931, **12,** 513.
13. P. Kubelka, *J. Opt. Soc. Am.*, 1948, **38,** 448.

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ACTIVATION ANALYSIS FOR MERCURY IN BIOLOGICAL SAMPLES AT NANOGRAM LEVEL

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Summary—A new method has been devised for determining mercury in samples of biological origin. It is based on complete ignition of the sample in a silica tube, trapping volatile interfering activities such as bromine or chlorine, and selectively adsorbing mercury on a strip of filter paper which has been previously impregnated with elemental selenium. This strip is later counted for quantitative evaluation. The versatility of the method has been demonstrated by the analysis of a wide range of samples such as water, cellulose, flour, fish solubles or animal blood samples with mercury contents between 1 and 200 ng/g of sample.

WIDESPREAD use of mercury-containing pesticides and fungicides makes it desirable to have a reliable picture of present and future background levels in the biosphere.

From the data collected by Goldwater[1] as well as from those reported later in the literature, the concentration of mercury in "normal" plants, fruits, vegetables or biological fluids could be expected to lie between a few hundred and less than one ng/g. Considerable fluctations can be expected, however, depending on the character of the soil.

The volatility of elemental mercury and many of its compounds presents a serious contamination problem in analysis for mercury at ng level. The techniques sensitive enough are atomic absorption[2,3,4] or fluorescence,[5] and activation analysis.[6–10] The last-named can be carried out in such a way as to eliminate the effect of possible contamination during processing and is therefore advantageous.

Non-destructive analysis proved successful[7] in analysis of simple systems for the upper levels of the concentration range given above. Considerable cooling periods are usually required which have frequently led in the past to basing the analysis on 47-day ^{203}Hg, whereas the 65-hr ^{197}Hg offers 100-fold improvement in sensitivity.

In general, in the case of biological materials, mercury is isolated from solutions resulting from the wet-ashing of samples. The presence of interfering activities in the activated samples, such as halogens, sodium, potassium, copper and arsenic, requires high decontamination factors for acceptable precision to be obtained in the evaluation of spectra.

Heterogeneous exchange between mercury in solution and elemental mercury[11] added in large excess has found application as a method for recovery of activated mercury. Two drawbacks of this method are the reduced counting efficiency due to self-absorption of the 68–77 keV ^{197}Hg peak and the necessity of using relatively large quantities of mercury in a laboratory engaged in low-level work. The alternative method based on the separation of mercury by electrolysis also gives high decontamination factors but in both methods difficulties arise in achieving the required purity for satisfactory precision at the 1-ng level.

A modified dithizone extraction procedure as used in this laboratory has been shown to give high decontamination factors for mercury. It has the added advantage that only μg amounts of carrier are required. The excess of reagent also ensures efficient removal of mercury from the laboratory glassware, thus reducing the contamination problem.

Isolation of activated mercury by volatilization

The method proposed here is based on complete volatilization of mercury during ignition of the organic substance. Volatile compounds formed during thermal

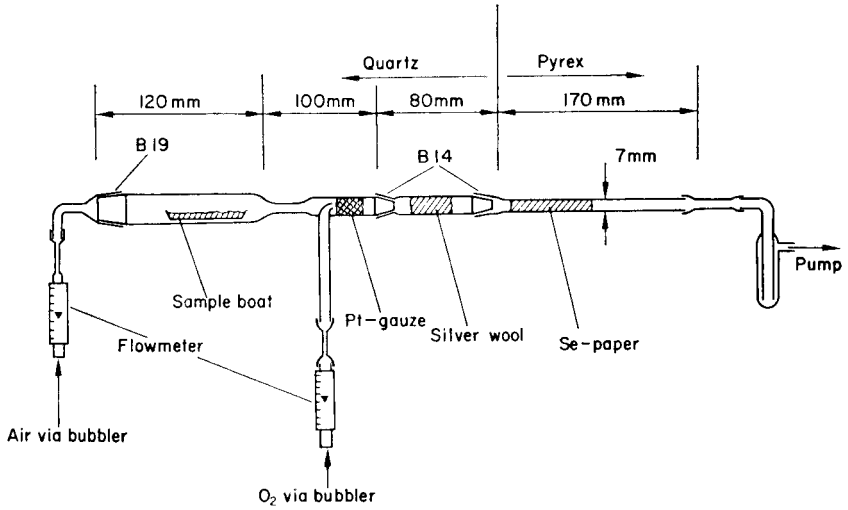

FIG. 1.—Apparatus for the separation of activated mercury by volatilization (furnaces not shown).

decomposition of organic substances are carried by a stream of air over a heated platinum gauze. A slow stream of oxygen is admitted into the system at this point in order to ensure complete ignition to carbon dioxide and water.

Volatile activities such as halogens, selenium and arsenic are retained by a pad of silica wool on which silver has been deposited. Mercury passes this trap to be adsorbed finally on a piece of selenium paper. The use of selenium as an adsorbent for mercury was described by Stitt and Tomimatsu.[12] Recovery of mercury has been shown to be quantitative in the presence of a few tens of μg of carrier. In about 200 ignitions carried out so far there was never any other activity on the selenium paper but that of pure mercury.

EXPERIMENTAL

Apparatus

A general view of the apparatus is shown in Fig. 1. The combustion tube and the tube containing the silver-coated quartz wool are made of silica. The tube containing the selenium-impregnated paper is Pyrex. Two bubble-traps and flow-meters monitor the rate of entry of air and oxygen: the flow is maintained by a ballasted water-pump. The sample boat has overall dimensions of 75 × 12 × 9 mm depth (capacity about 4 ml).

Reagents

Silver-coated quartz wool. Moisten 3–4 g of silica wool with an ammoniacal solution of silver nitrate made from 25 ml of 0·2*M* silver nitrate, and add an equal volume of 10% sodium tartrate solution. The wool is left until the formation of the silver mirror is complete. It is then thoroughly washed and dried. This procedure gives enough material for about 10 combustions.

Selenium-impregnated paper. Shake a small excess of powdered black selenium in 0·2*M* sodium cyanide solution. Wide-pore filter paper such as Schleicher & Schüll No. 589[1] is dipped into the solution, drained, then dipped into hydrochloric acid (1 + 1) and finally thoroughly washed with water. It is then dried under an infrared lamp to produce a paper with a reddish-orange colour. For use, the paper is cut into 50-mm squares.

Dithizone solution. Dissolve 10 mg of reagent quality dithizone in 100 ml of carbon tetrachloride and store in the dark.

Irradiation

Solid samples were sealed inside polyethylene capsules of 12 mm diameter and 8 ml capacity, supplied by Medisciences, Paris (Hémo-etran tubes). They were exposed to an integrated flux of 1·0–2·0 × 10^{15} n/mm²/sec in the irradiation facility of the Institute's reactor Triga Mark II. Standards were in the form of solution which was weighed into silica tubes (polyethylene appears to adsorb mercury strongly and is not suitable for the purpose).

Counting equipment included a Nuclear Data, ND-180, 512-channel γ-spectrometer. In fact, however, the separation procedure is so specific for mercury that essentially the same values were obtained from the total count rates.

Ignition of the sample

The irradiation sample is loaded into the silica boat and a small amount of carrier added. Some of the silvered silica wool is packed into the section of the silica tube following the platinum gauze. A fairly firm pad about 35 mm long is formed.

A 50-mm square of selenium paper is rolled into a tight cylinder and inserted into the final adsorption tube. The apparatus is assembled and the furnaces around the silvered quartz wool and selenium paper are switched on, while a slow stream of air is passed through the apparatus. After a few minutes the furnaces will have attained their equilibrium temperatures of 300° and 100° respectively. The oxygen flow is then started at 0·2 l./min, the air flow being about 0·1 l/min. A Bunsen burner is placed below the platinum gauze, maintaining it at red heat. The furnace around the sample is then switched on. The temperature reaches 400° in about 15 min and is held there for a further 5 min. The gases burn quietly on the platinum gauze. In a few hundred combustions no explosion or flashback has been observed, and no tar or smoke has been produced in the ignition, which gives only oxides of carbon, nitrogen and water.

The ignition is by now complete so the furnaces are switched off and the Bunsen removed. The mercury adsorption tube is detached from the apparatus, the selenium paper withdrawn and counted in the multichannel analyser.

In a slightly modified version of the apparatus liquids can also be analysed. The principle also proved very useful for studying losses of mercury by volatilization from various media.

Standards

Solutions were prepared by dissolving reagent quality mercury(II) oxide in nitric acid and diluting the solution in calibrated volumetric equipment to a concentration of 1 μg of mercury/ml. The final solution was measured into the quartz tubes by weight. These were then irradiated in contact with the polythene sample containers, the height of the liquid being adjusted to that of the sample. After irradiation they were opened and 10 μg of mercury added. The solution was quantitatively transferred to an extraction funnel, with several washings with 1*M* sulphuric acid.

Two extractions with 2 ml of the dithizone solution were carried out and the volume of the extract made up to the mark with carbon tetrachloride after transfer to a 5-ml volumetric flask. It has been shown by tracer experiments that this procedure gives over 99·9% extraction of the mercury. For counting, a 1-ml aliquot is transferred to a glass vial which fits into the well of the 75-mm sodium iodide crystal.

RESULTS

Recovery of mercury

Further tracer experiments with mercury demonstrated that the recovery of mercury during ignition of the sample is 99–100%, provided that μg quantities of mercury

are present. When the weight of mercury in the tracer was in the range 0·2–1·0 μg, the yields attained were proportionately 80–97%.

The carrier needed for quantitative recovery was added to the sample, in the form of a paper spotted with the carrier solution, dried and placed on the bottom of the silica boat.

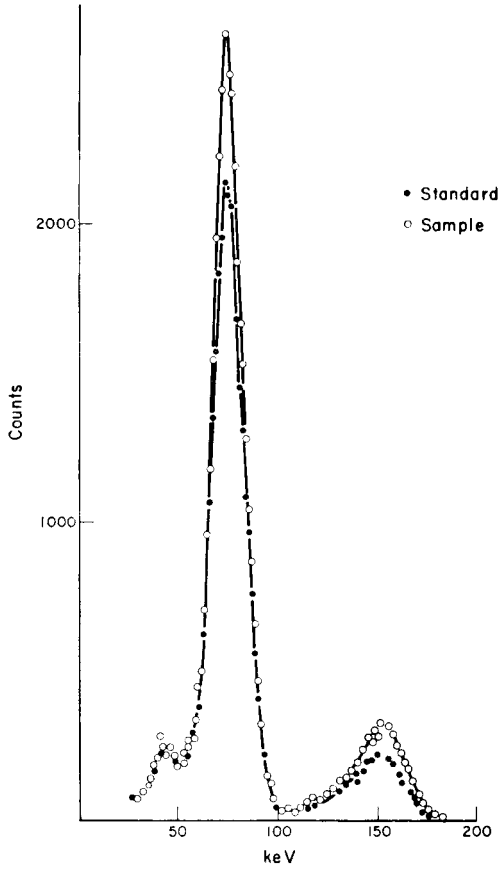

FIG. 2.—γ-Spectra of separated mercury on selenium paper from ignition of grass sample and of mercury standard. Mercury content 140 ng/g; counting period 4 min.

Radiochemical purity

The combination of silver and selenium has been proved to give pure mercury activity free from any contamination even at levels below 1 ng/g. Figures 2 and 3 show typical spectra obtained by our method for samples with the highest and lowest mercury contents of the materials examined. Even very active samples rich in bromine, phosphorus and arsenic, such as fish solubles or animal blood serum, gave pure mercury spectra on immediate processing after irradiation when surface doses were of the order of 1 r/hr. Thus silver represents a highly efficient filter for volatile activities other than mercury. We have never observed any other activity on the selenium or in the scrub solutions following the selenium paper. On the other hand the trapping efficiency of this paper for mercury is very high. In fact 95% of the activity is found on the first 5 mm of the roll and the rest within the next 5 mm.

Precision and accuracy

The precision of the method was demonstrated by the multiple analysis (usually 0·5-g samples) of a wide selection of biological samples. The results are summarized in Table I.

The first six samples were made available by the courtesy of the International Atomic Energy Agency laboratory in Seibersdorf, Austria. The kale is standard reference material kindly supplied by Bowen.[13] The mercury content of this kale is 0·175 μg/g (mean value of 27 determinations by activation analysis from 7 different

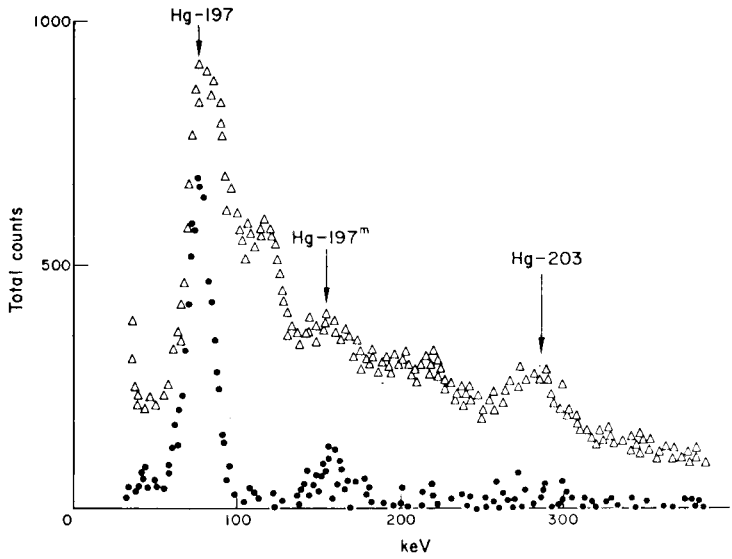

FIG. 3.—γ-Spectra of selenium paper from ignition of 0·5 g of wheat flour with and without background subtraction. Mercury content 2·4 ng/g, counting period 80 min, integrated flux $1{\cdot}5 \times 10^{17}$ n/cm^2.

laboratories[14]). Results are expressed in terms of sample dry weight. Drying conditions were 20 hr at 90°, in accordance with the recommendations of Bowen.[13]

Cellulose and sawdust were also analysed by a modified dithizone extraction procedure and found to contain 96 and 27 μg/g respectively in good agreement with the volatilization method. This comparative procedure however is not satisfactory for samples low in mercury and rich in certain contaminants. Bromine especially could not be entirely removed by extraction. Precision of the volatilization method does not deteriorate even at amounts of mercury of the order of 1 ng. Sample weights were normally 0·5 g. The precision in terms of the coefficient of variation is about 5% in the range of mercury concentration between 1 and 200 ng/g.

Flux variation across the annular irradiation facility of the Triga reactor was found to be less then 2%. As mentioned above, the standards were in contact with the sample and of the same height so that the dose received should be identical.

A systematic error could be introduced, however, through slightly different geometry and absorption factors between the selenium paper and the standard dissolved in 1 ml of carbon tetrachloride. Therefore the selenium paper was introduced into the counting vial in a standard manner so as to occupy approximately 1 ml.

TABLE I.—CONCENTRATION OF MERCURY FOUND IN SOME TYPICAL BIOLOGICAL MATERIALS

Sample	No. of aliquots	Results, *ng/g*	Mean, *ng/g*	Coeff. of variation, %
Cellulose	3	91·9, 95·8, 92·3	93·3	2
Fish solubles	4	76·6, 73·4, 74·2, 68·5	73·2	4
Sawdust	3	29·6, 29·1, 28·4	29·0	2
Animal blood	4	13·8, 12·5, 12·5, 11·8	12·7	6·5
serum	4	11·9, 12·8, 12·1, 11·5	12·1*	4
Corn flour	5	3·1, 3·0, 3·6, 2·7, 3·3	3·15	10
Wheat flour	5	2·5, 2·6, 2·4, 2·5, 2·7	2·5	5
Grass	4	130, 135, 146, 138	137	5
River water	2	0·061, 0·065	0·063	
Kale	9	160, 175, 167, 180, 179, 164, 176, 166, 166	170	4

* Results for this independent set of determinations were obtained from the total count rate.

The relative counting efficiencies were determined by measuring first the activity of the tip of a paper spotted with mercury(II) dithizonate and again after adding 1 ml of carbon tetrachloride. The difference in efficiencies was found to be 7% and this correction factor was applied to all measurements.

Further applications

With a large furnace tube and boat, and a water trap in the gas train behind the selenium paper, liquid samples (10–100 ml) such as urine or industrial effluents, can be analysed. The liquid is gently evaporated through the system and the residue ignited.

Two samples (6 ml each) of a local river water were analysed. With a counting period of 5 hr the mercury content was shown to be as little as 63 pg/ml.

The method has several advantages: rapidity; no chemical processing; no yield determination; processing can be begun immediately after irradiation, local screens being used round the combustion tubes if sodium and potassium levels are high; excellent radiochemical purity; suitable for ng-levels.

Zusammenfassung—Ein neues Verfahren zur Bestimmung von Quecksilber in Proben biologischen Ursprungs wurde entwickelt. Es beruht auf vollständiger Veraschung der Probe in einem Quarzrohr, dem Auffangen störender flüchtiger Aktivitäten wie Brom oder Chlor und selektiver Adsorption von Quecksilber an einem Streifen Filterpapier, der vorher mit elementarem Selen imprägniert wurde. Zur quantitativen Bestimmung werden die Impulse dieses Streifens nachher gezählt. Die Vielseitigkeit der Methode wurde demonstriert an der Analyse von vielen verschiedenen Proben wie Wasser, Cellulose, Mehl, löslichen Fischbestandteilen und Tierblut mit Quecksilbergehalten zwischen 1 und 200 ng/g Probe.

Résumé—On a établi une nouvelle méthode pour déterminer le mercure dans des échantillons d'origine biologique. Elle est basés sur la calcination complète de l'échantillon dans un tube de silice, piégeant les activités volatiles interférantes, comme le brome ou le chlore, et absorbant sélectivement le mercure sur une bande de papier filtre que l'on a préalablement imprégnée de sélénium élémentaire. Cette bande est ensuite soumise au comptage pour évaluation quantitative. On a démontré le caractère général de la méthode par l'analyse d'une large gamme de composés tels que l'eau, la cellulose, la farine, des extraits solubles de poisson ou des échantillons de sang animal avec des teneurs en mercure comprises entre 1 et 200 μg par g d'échantillon

REFERENCES

1. L. J. Goldwater, *The Harben Lectures 1964*, Royal Institute of Health and Public Hygiene.
2. O. Lindström, *Anal. Chem.* 1959, **31,** 461.
3. J. B. Willis, *ibid.*, 1962, **34,** 615.
4. E. Berman, *At. Absorption Newsletter*, 1967, **6,** 67.
5. G. Thilliez, *Chim. Anal.*, 1968, **50,** 226.
6. T. J. Vickers and S. P. Merrick, *Talanta*, 1968, **15,** 873.
7. T. Westermark and B. Sjöstrand, *Intern. J. Appl. Radiation Isotopes*, 1960, **9,** 1.
8. B. Sjöstrand, *Anal. Chem.* 1964, **36,** 814.
9. D. Comar, *J. Lab. Clin. Med.* 1965, **66,** 168.
10. R. Christell, L. G. Erwall, K. Ljunggren, B. Sjöstrand and T. Westermark, *Proc. 1965 Int. Conf. Modern Trends in Act. Analysis*, College Station, Texas, 1966, 380.
11. C. K. Kim and T. Silverman, *Anal. Chem.* 1965, **37,** 1616.
12. F. Stitt and Y. Tomimatsu, *ibid.*, 1951, **23,** 1098.
13. H. J. M. Bowen, *Analyst*, 1967, **92,** 124.
14. *Idem*, private communication.

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DETERMINATION OF COBALT AND ZINC IN HIGH-PURITY NIOBIUM, TANTALUM, MOLYBDENUM AND TUNGSTEN METALS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY AFTER SEPARATION BY EXTRACTION

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Summary—A method for determining 0·0005–0·05% of cobalt and zinc in high-purity niobium, tantalum, molybdenum and tungsten metals by atomic-absorption spectrophotometry is described. After sample dissolution, cobalt and zinc are separated simultaneously from the matrix materials by chloroform extraction of their thiocyanate–diantipyrylmethane ion-association complexes, at pH 3·25, from a citric acid medium approximately 1·2*M* in sodium thiocyanate. Interference from copper is eliminated with thiourea. Large amounts of iron interfere under the recommended conditions, but moderate amounts may be present in the sample solution without causing appreciable error in the results. Phosphorus (as orthophosphate) interferes in the extraction of cobalt from tungsten solutions. Moderate amounts of other impurities do not interfere in the proposed method.

RECENTLY, a method for determining trace amounts of cobalt and zinc in nickel metal by atomic-absorption spectrophotometry[1] was developed in this laboratory. In this method, which was based on a procedure previously described by Jankovský,[2] cobalt and zinc are separated simultaneously from the matrix element by chloroform extraction of their thiocyanate-diantipyrylmethane complexes, and interference from copper and various other elements is eliminated with thiourea and ammonium fluoride. The present investigation was undertaken because it was considered that, with some modifications, this method could probably be applied to high-purity niobium, tantalum, molybdenum and tungsten metals.

Various wet-chemical methods involving spectrophotometric,[3–8] spectrographic,[9–11] polarographic,[8,12] neutron activation[13,14] and atomic-absorption spectrophotometric[15] finishes have previously been applied to the determination of small amounts of cobalt and/or zinc in these metals, but no single method has hitherto been reported for determining both elements in all four of them.

The procedure used for sample dissolution is essentially that previously reported in methods for determining other impurities.[16–19] Under the proposed conditions, the formation and extraction of the thiocyanate-diantipyrylmethane complexes of the quinquevalent metal ions mentioned, particularly niobium and tantalum, is largely prevented or inhibited. Small amounts of molybdenum and tungsten are co-extracted but do not interfere in the determination of cobalt and zinc. Iron is partially extracted, but moderate amounts may be present in the sample solution without causing excessive error in the results. More than about 0·5 mg of phosphorous (as orthophosphate) interferes in the extraction of cobalt from tungsten solutions.

EXPERIMENTAL

Apparatus

Techtron Model AA-3 atomic-absorption spectrophotometer equipped with a recorder, a 100-mm laminar-flow air–acetylene burner and cobalt and zinc hollow-cathode lamps.

Instrument settings for zinc: wavelength 213·9 nm; lamp current 6 mA; slitwidth 300 μm; air pressure 180 kN/m^2; acetylene pressure 17·2 kN/m^2.

Instrument settings for cobalt: wavelength 240·7 nm; lamp current 10 mA; slitwidth 100 μm; air pressure 180 kN/m^2; acetylene pressure 17·2 kN/m^2.

Reagents

Standard cobalt solution, 200 ppm. Dissolve 0·8075 g of cobalt(II) chloride hexahydrate in water and dilute to 1 litre. Prepare working solutions approximately 0·1*M* in hydrochloric acid and containing from 1 to 6 ppm of cobalt.

Standard zinc solution, 200 ppm. Dissolve 0·2000 g of pure zinc metal in 20 ml of 2*M* hydrochloric acid, evaporate the solution to dryness, dissolve the residue in water and dilute to 1 litre. Prepare working solutions approximately 0·1*M* in hydrochloric acid and containing from 0·20 to 0·80 ppm of zinc.

Diantipyrylmethane. 2% w/v solution in ethanol.

Ammonium chloride–ammonia buffer solution. 2·5% w/v and v/v, respectively.

Procedure

Run a reagent blank along with the samples. Transfer a 1-g sample (weighed to 1 mg) of the powdered metal (Nb, Ta, Mo or W) to a 250-ml Teflon beaker, add 5 ml of water and 4 ml of hydrofluoric acid (plastic pipette) and cover the beaker with a Teflon cover. Then add 2 ml of concentrated nitric acid and heat gently on the hot-plate until all of the metal is in solution. (For molybdenum metal, add at this point 3 ml of concentrated hydrochloric acid and heat until the dark brown colour disappears and the solution becomes pale yellow). Add 5 ml of formic acid to destroy excess of nitric acid and heat gently until the evolution of brown oxides of nitrogen ceases. Remove the cover, wash down the sides of the beaker with a small amount of water and evaporate the solution to about 5 ml. Add 10 ml of 50% citric acid solution and 40 ml of 5% boric acid solution, mix, allow to stand for 15 min (Note 1) and then adjust the pH to 3·25 $\pm$ 0·10 with concentrated ammonia solution. After allowing 15 min for the sample solution to cool to room temperature, add 3 ml of 10% thiourea solution and 30 ml of 50% sodium thiocyanate solution. Mix well after each addition and readjust the pH to exactly 3·25. Transfer the resulting solution to a 250-ml separatory funnel and dilute to approximately 150 ml with water. Add 5 ml of 2% diantipyrylmethane solution, mix, then add 10 ml of chloroform, stopper and shake for 1 min. Allow several minutes for the layers to separate, then drain the chloroform extract into a 60-ml separatory funnel. Extract the sample solution three more times (depending on the cobalt and zinc contents of the sample) using, in succession, 5, 3 and 2 ml of diantipyrylmethane solution and 5 ml of chloroform each time (Note 2). Combine the extracts, add 20 ml of ammonia buffer solution, shake for 30 sec, allow the layers to separate and discard the chloroform layer. Transfer the aqueous layer containing the cobalt and zinc to a 100-ml beaker, add 5 ml of concentrated hydrochloric acid, heat gently to remove any chloroform, and then evaporate the solution to about 15 ml. Cover the beaker with a watch glass, add 5 ml each of concentrated hydrochloric and nitric acids and boil to destroy ammonium salts and organic material. Remove the cover and gently evaporate the solution to dryness on the hot-plate. Add 2 ml of concentrated hydrochloric acid and 5 ml of water to the residue in the beaker and evaporate the solution to dryness in a water-bath (Note 3). Then add about 10 ml of 0·1*M* hydrochloric acid and heat gently to dissolve the cobalt and zinc salts. Filter into a 25-ml volumetric flask, using the 0·1*M* hydrochloric acid for washing and diluting to volume (Note 4). Analyse this solution (or a suitable dilution of it with 0·1*M* hydrochloric acid) (Note 5) for cobalt and zinc by measuring their atomic absorptions under the appropriate instrumental conditions and relating the values to those obtained concurrently for standards of slightly higher and lower concentrations. Correct for the reagent blank.

Notes

1. Niobium, tantalum and molybdenum solutions may be allowed to stand overnight at this point. However, tungsten solutions should be extracted soon after preparation because prolonged standing sometimes results in precipitation of a yellow compound (presumably hydrated tungsten trioxide).

2. Because of the solubility of diantipyrylmethane in chloroform, more must be added before each extraction step to complex any cobalt and zinc remaining in the aqueous phase. Extraction of

both cations is complete when the blue colour of the cobalt complex no longer forms in the aqueous phase on addition of diantipyrylmethane. Fifteen ml of 2% diantipyrylmethane solution are usually sufficient for 0·5 mg each of cobalt and zinc.

3. Excessive heating of cobalt salts results in the formation of black Co_3O_4 which is relatively insoluble in water or dilute hydrochloric acid, causing low results. This difficulty is avoided by dissolution of the oxides with concentrated hydrochloric acid followed by evaporation of the resultant solution to dryness in a water-bath.

4. Both test and standard solutions are made up to the same acidity because it was found previously[1] that differences in their chloride content caused slightly high results for cobalt and zinc.

5. Calibration curves for zinc and cobalt are linear and approximately linear, respectively, in the concentration ranges 0–0·80 ppm of zinc and 0–6 ppm of cobalt under the recommended conditions; dilutions are made accordingly.

RESULTS

Effect of pH

In the previous investigation[1] it was established that extraction of the cobalt and zinc is complete at pH 1·40–3·45. Because the masking agent (ammonium fluoride) used by Jankovský[2] to suppress iron(III) interference was found ineffective in this pH range, extraction at pH 3·25 from citric acid medium was used earlier and again now. Although small amounts of iron(III) (5 mg) are not complexed completely by citric acid at this pH, the amount co-extracted into chloroform does not interfere.

Effect of sodium thiocyanate

Preliminary experiments showed that the amount of sodium thiocyanate (5 g) used in the previous method[1] was not sufficient for complete extraction of cobalt (500 μg) in the present investigation, although adequate for extraction of 500 μg of zinc. It was found that 15 g were needed for complete extraction of cobalt.

Effect of diverse ions

It was previously found that iron(III) and cadmium are partially extracted at pH 3·25 from solutions containing citric acid, ammonium fluoride and thiourea. Large amounts of chromium(III) interfere with the extraction by partial precipitation, probably as chromium(III) fluoride, which causes emulsification in the chloroform phase. However, moderate amounts of these (5, 10 and 10 mg respectively) and at least 10-mg amounts of various other ions [copper(II), lead, manganese(II), vanadium(V), zirconium, titanium(IV), bismuth, tin(IV), antimony(V), chromium(VI), arsenic(V) and phosphorus(V)] did not interfere in the determination of cobalt and zinc.[1]

Although most of these ions would not be expected to interfere in the present work, particularly in niobium and tantalum solutions, several could interfere in molybdenum and tungsten solutions by forming heteropoly compounds with these elements. Other ions could, on reduction with thiourea, cause the catalytic reduction and subsequent extraction of some of the matrix element. Consequently, it was necessary to investigate those ions which could interfere in these ways. The results are given in Table I, which shows that for the quantity of each ion tested none affected the extraction of zinc, but iron in molybdenum solutions and both iron and orthophosphate in tungsten solutions interfered with the extraction of cobalt. However, at the 500-μg level for cobalt, up to 0·5 mg of phosphorus(V) can be tolerated in tungsten solutions, and up to 4 and 3 mg of iron in molybdenum and tungsten solutions, respectively. Some molybdenum and tungsten were extracted into the chloroform phase with all of the ions tested, but do not affect the atomic absorption.

TABLE I.—EFFECT OF DIVERSE IONS ON THE EXTRACTION OF COBALT AND ZINC FROM MOLYBDENUM AND TUNGSTEN SOLUTIONS

Test solution	Co and Zn taken, μg	Co found, μg	Zn found,* μg	Elements co-extracted with Co and Zn		
				Mo found, *mg*	W found, *mg*	Fe found, *mg*
Mo + 5 mg Cu(II)	250	251	248	0·13	—	—
Mo + 5 mg V(V)	250	251	250	0·13	—	—
Mo + 5 mg Ti(IV)	250	251	252	0·09	—	—
Mo + 5 mg Cr(VI)	250	251	246	0·22	—	—
Mo + 5 mg As(V)	250	250	242	0·24	—	—
Mo + 5 mg P(V)	500	502	499	1·18	—	—
Mo + 5 mg Fe(III)	500	481	502	0·78	—	0·94
Mo + 4 mg Fe(III)	500	498	499	0·42	—	1·45
W + 5 mg Cu(II)	250	251	252	—	0·46	—
W + 5 mg V(V)	250	252	247	—	0·67	—
W + 5 mg Ti(IV)	250	249	252	—	0·55	—
W + 5 mg Cr(VI)	250	251	246	—	0·76	—
W + 5 mg As(V)	250	250	247	—	0·82	—
W + 2 mg P(V)	500	453	500	—	1·39	—
W + 1 mg P(V)	500	485	500	—	1·86	—
W + 0·5 mg P(V)	500	498	500	—	1·69	—
W + 4 mg Fe(III)	500	486	499	—	0·93	0·30
W + 3 mg Fe(III)	500	500	501	—	1·28	0·81

* Corrected for the zinc contents of the respective metals (Table II).

However, larger amounts were co-extracted in the presence of iron and phosphorus, suggesting some reduction of the matrix elements and the formation and partial extraction of heteropoly compounds, respectively. Compound formation in tungsten solutions was observed when about 3 mg or more of phosphorous(V) were present. The solution became decidedly milky in appearance during the initial pH adjustment and a white, insoluble compound appeared in the chloroform during the extraction.

Several additional tests showed that 5 mg of iron(III) and phosphorus(V) do not affect the extraction of cobalt and zinc from niobium and tantalum solutions.

Accuracy

The proposed method was applied to the analysis of a series of synthetic samples in which the added cobalt and zinc were both varied from 0·001 to 0·05%. The standard cobalt and zinc solutions were added directly to the powdered metal samples. The results are given in Table II, and show good recoveries.

DISCUSSION

With the exception of phosphorous(V) in tungsten solutions, iron is the most serious interferent in the extraction of cobalt because it is not completely complexed with citric acid under the proposed conditions. The uncomplexed portion reacts with sodium thiocyanate and diantipyrylmethane to form a reddish-brown complex, which is destroyed, to some extent, on shaking with chloroform. If large amounts are present in the sample solution, the colour obscures the characteristic colour of the cobalt complex, making it difficult to determine when extraction is complete. In general, more iron is co-extracted, particularly from molybdenum and tungsten solutions, than in the previous investigation[1] (<100 μg); more is extracted from tantalum than from niobium solutions (0·58 and 0·05 mg, respectively in the presence of 500 μg each of cobalt and zinc). This increased extraction could be caused by the increase in the sodium thiocyanate concentration.

TABLE II.—RECOVERY OF COBALT AND ZINC BY THE PROPOSED METHOD FROM SYNTHETIC NIOBIUM, TANTALUM, MOLYBDENUM AND TUNGSTEN SAMPLES

Matrix	Total Co present, %	Co found, %	Total Zn present, %	Zn found, %
Nb	0·0020	0·0021	0·0012	0·0011
	0·0060	0·0061	0·0052	0·0048
	0·0110	0·0111	0·0102	0·0099
	0·0260	0·0260	0·0252	0·0249
	0·0510	0·0515	0·0502	0·0505
Ta	0·0010	0·0010	0·0011	0·0009
	0·0050	0·0049	0·0051	0·0051
	0·0100	0·0100	0·0101	0·0103
	0·0250	0·0250	0·0251	0·0248
	0·0500	0·0507	0·0501	0·0504
Mo	0·0010	0·0010	0·0012	0·0011
	0·0050	0·0049	0·0052	0·0051
	0·0100	0·0099	0·0102	0·0101
	0·0250	0·0252	0·0252	0·0253
	0·0500	0·0501	0·0502	0·0502
W	0·0010	0·0010	0·0011	0·0010
	0·0050	0·0050	0·0051	0·0049
	0·0100	0·0100	0·0101	0·0100
	0·0250	0·0249	0·0251	0·0248
	0·0500	0·0498	0·0501	0·0501

Duplicate determinations of cobalt and zinc in the Nb, Ta, Mo and W metals by the proposed method gave average results of 0·0010%, nil, nil, nil respectively for cobalt, and 0·0002%, 0·0001%, 0·0002%, 0·0001% respectively for zinc.

Whereas 15 ml of 2% diantipyrylmethane solution suffice for extraction of 1 mg each of cobalt and zinc from nickel solutions,[1] here this amount is required for 0·5 mg each of these elements, presumably because of the co-extraction of some of the matrix materials and their inhibiting action (indicated by the amount of sodium thiocyanate required) on the extraction of cobalt.

Co-extraction of niobium and tantalum was not investigated, because no hydrolysis or hydroxide formation was observed in tests with these solutions when the chloroform extracts containing cobalt and zinc were shaken up with the ammonia buffer.

The method is suitable for samples containing 0·0005–0·05% of cobalt and zinc. Although the sensitivity is much higher for zinc than for cobalt, this may be offset by the high zinc content of the reagent blank (approximately 10 μg in our experiments).

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Zusammenfassung—Eine Methode zur Bestimmung von 0,005–0,05% Kobalt und Zink in hochreinem Niob, Tantal, Molybdän und Wolfram durch Atomabsorptionsspektrophotometrie wird beschrieben. Nach Auflösen der Probe werden Kobalt und Zink zusammen abgetrennt, indem die Ionenassoziate ihrer Thiocyanate mit Diantipyrylmethan bei pH 3,25 aus einem Zitronensäuremedium, das etwa 1,2*M* Natriumthiocyanat enthält, mit Chloroform extrahiert werden. Die Störung durch Kupfer wird mit Thioharnstoff beseitigt. Große Mengen Eisen stören unter den empfohlenen Bedingungen; mäßige Mengen können ohne wesentliche Verfälschung der Ergebnisse in der Probenlösung vorhanden sein. Phosphor (als Orthophosphat) stört die Extraktion von Kobalt aus Wolframlösungen. Mäßige Mengen anderer Verunreinigungen stören bei der vorgeschlagenen Methode nicht.

Résumé—On décrit une méthode de détermination de 0,005–0,05% de cobalt et de zinc dans les niobium, tantale, molybdène et tungstène métalliques de haute pureté par spectrophotométrie d'absorption atomique. Après dissolution de l'échantillon, on sépare simultanément le cobalt et le zinc des substances de la matrice par extraction au chloroforme de leurs complexes d'association ionique thiocyanate–diantipyrylméthane, à pH 3,25, à partir d'un milieu acide citrique approximativement 1,2*M* en thiocyanate de sodium. On élimine l'interférence du cuivre par la thiourée. De fortes quantités de fer interfèrent dans les conditions recommandées, mais des quantités peu élevées peuvent être présentes dans la solution échantillon sans causer d'erreur appréciable dans les résultats. Le phosphore (à l'état d'orthophosphate) interfère dans l'extraction du cobalt de solutions de tungstène. Des quantités peu élevées d'autres impuretés n'interfèrent pas dans la méthode proposée.

REFERENCES

1. E. M. Donaldson and V. H. E. Rolko, Dept of Energy, Mines and Resources, Mines Branch, Ottawa, Technical Bulletin TB 93, August 1967.
2. J. Jankovský, *Z. Anal. Chem.*, 1965, **213,** 84.
3. T. K. Marshall, J. W. Dahlby and G. R. Waterbury, *U.S. At. Energy Comm. Rept.*, LA-3124, 1964.
4. G. Norwitz and H. Gordon, *Anal. Chem.*, 1965, **37,** 417.
5. O. Grossmann and H. Grosse-Ruyken, *Z. Anal. Chem.*, 1967, **233, 14.**
6. R. Puschel, E. Lassner and A. Illaszewicz, *Chemist-Analyst*, 1966, **55,** 40.
7. V. A. Nazarenko, S. A. Biryuk, M. B. Shustova, G. G. Shitareva, S. Ya. Vinkovetskaya and G. V. Flyantikova, *Zavodsk. Lab.*, 1966, **32,** 267.
8. C. Tombu, *Tantale, Niobium, Analyses des Produits Pure Oxyde et Metal*, Société Générale Métallurgique de Hoboken, Laboratory Manual, 1961.
9. G. L. Hubbard and T. E. Green, *Anal. Chem.*, 1966, **38,** 428.
10. N. N. Kuznetsova and L. S. Krauz, *Zh. Analit. Khim.*, 1963, **18, 1090.**
11. E. A. Huff, *Anal. Chem.*, 1964, **36,** 1921.
12. J. Doležal and J. Novák, *Collection Czech. Chem. Commun.*, 1958, **23,** 866.
13. M. Eychenne, *Chim. Anal.*, 1967, **49,** 355.
14. G. Pinte, *Application of Neutron Activation Analysis to the Study of Impurities in Molybdenum Tungsten and Nuclear Graphite*, Commissariat à l'Énergie Atomique, Centre d'Études Nucléaires, Soclay, France, October 1967.
15. J. H. Muntz and L. L. Roush, *U.S. At. Energy Comm. Accession*, No. 43154, *Rept.* No. AED-CONF-66-172-9, 1966.
16. E. M. (Penner) Donaldson and W. R. Inman, *Talanta*, 1966, **13,** 489.
17. E. M. Penner and W. R. Inman, *ibid.*, 1962, **9,** 1027.
18. *Idem, ibid.*, 1963, **10,** 407.
19. *Idem, ibid.*, 1963, **10,** 997.

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COULOMETRIC TITRATION OF BASES IN ACETIC ACID AND ACETONITRILE MEDIA

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Summary—The working conditions and the results for coulometric titration of milligram amounts of some bases in 0.1*M* sodium perchlorate in a mixture of acetic acid and acetic anhydride (1:6), are given. Determinations were made both by coulometric back-titration or direct titration at the platinum anode. Back-titration was done in the catholyte, by coulometric titration of the excess of added perchloric acid. The titration end-point was detected photometrically with Crystal Violet as indicator. The direct titration of bases was done at the platinum anode, in the same electrolyte, to which hydroquinone was added as anode depolarizer and as the source of hydrogen ions, Malachite Green being used as indicator. Similarly, bases can be determined in acetonitrile if sodium perchlorate, hydroquinone and Malachite Green are added to the solvent. Errors are below 1%, and the precision is satisfactory.

DETERMINATION of bases in acetic acid and acetonitrile by volumetric and potentiometric titration has found extensive application. Coulometric titration of these systems has also been described,[1,2] but in acetonitrile is applicable only to stronger bases, and in acetic acid only to tertiary amines and salts of organic acids.

Direct coulometric titration of bases at a platinum anode in pure acetic acid or in a mixture of acetic acid and acetic anhydride is avoided owing to the oxidation[3] of acetate ion (this is the basis of Kolbe's hydrocarbon synthesis). Though in this oxidation hydrogen ions should be generated at a current efficiency of 100%, Mather and Anson[2] obtained a maximum of only 95%, because of secondary processes at the anode. They therefore used a mercury anode, which gave 100% current efficiency in generation of hydrogen ions although several processes occur simultaneously at this anode. According to Durand and Trémillon,[4] at the anode mercury is primarily oxidized to mercury(I), which subsequently combines with acetate to form sparingly soluble mercury(I) acetate, the solution becoming acidic because of removal of acetate ions. Mather and Anson observed that in potentiometric titrations the potential changes very slowly just before the equivalence point; this is probably connected with the slow formation of the mercury(I) acetate precipitate.

This disadvantage of the mercury electrode can be avoided if the electrolysis takes place on a platinum electrode in the presence of hydroquinone, formic acid or acetaldehyde, as noticed by Mather and Anson. Therefore one of the aims of the work described here was to investigate the possibility of determining bases directly at a platinum electrode in acetic acid in the presence of an anodic depolarizer.

Coulometric determination of bases in acetonitrile directly at the anode cannot be accomplished since hydrogen ions are liberated only at high potentials.[5] Schmidt and Noack[6] found that during electrolysis of silver perchlorate in acetonitrile, perchloric acid is produced quantitatively at the platinum anode. This supporting

electrolyte is, however, unsuitable for coulometric titrations of bases since it is possible that some amines may be oxidized at the potential at which perchloric acid is produced. In addition, anions reacting with silver will interfere.

Streuli[1] developed a method for the coulometric titration of bases in acetonitrile, where water is used as anodic depolarizer and is introduced in the form of $LiClO_4 \cdot 3H_2O$. In the anode process water gives hydrogen ions and oxygen: $2H_2O - 4e \rightarrow 4H^+ + O_2$. The oxygen evolved can cause unwanted oxidation, *e.g.*, in coulometric titration of aromatic amines. In a later communication Hanselman and Streuli[7] attempted to eliminate the evolved oxygen by addition of hydroquinone as antioxidant. Their method is suitable for the determination of strong bases, where water, as a much weaker base, does not interfere if in smaller amounts. In acetonitrile, however, even very weak bases (*e.g.*, urea) can be determined if precautions are taken that traces of water are not present. Kolthoff, Chantooni and Bhowmik[8] used perchloric acid to titrate a large number of weak bases in acetonitrile potentiometrically or in the presence of visual indicators. Therefore we tried, by avoiding even traces of water and by using a suitable anode depolarizer, to develop a coulometric procedure to determine even weak bases in acetonitrile, detecting the titration end-point photometrically, since this method, according to Kolthoff and collaborators, is more sensitive than the potentiometric one.

EXPERIMENTAL

All chemicals used were of analytical grade, and the solvents were purified according to procedures given in the literature.[9,10] The 0·1*M* perchloric acid (used in the back-titration method) was standardized against potassium hydrogen phthalate or sodium acetate. The substances titrated were usually pharmaceutical products, used without further purification. Samples were weighed into 25 or 50-ml volumetric flasks and then dissolved in the appropriate solvent to give a concentration of 3–5 mg/ml. For each titration 0·80–1·50 ml of solution was taken by microburette. The supporting electrolyte was 0·1*M* sodium perchlorate in a mixture of acetic acid and acetic anhydride (1:6 v/v) or 0·25*M* sodium perchlorate in acetonitrile.

Coulometric titrations were performed in the apparatus shown in Fig. 1. A current stabilizer type STNS "Mihajlo Pupin" was used, which stabilized the current to ±0·1%. The current was checked

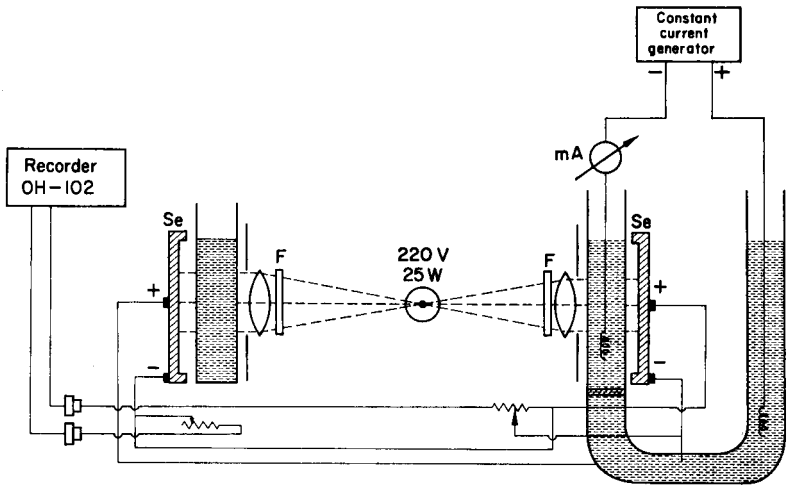

FIG. 1.—Scheme of the apparatus.
Se—selenium photocell; F—filter; reference solution in the left-hand cuvette; anolyte separated from catholyte by a sintered-glass disc, porosity 4.

by a high-precision milliammeter. The capacity of the electrolytic vessel in which the determinations were made was about 25 ml. The electrodes were 0·5-mm diameter platinum wire spirals with surface area of 270 mm^2, and the solution was vigorously stirred by means of a magnetic stirrer. The titration end-point was detected with a Lange universal colorimeter (Model VI); the reference solution was pure supporting electrolyte plus indicator.

Determination of bases in acetic acid solution

We first developed a procedure for continuously following photometrically the acidity of the solution during coulometric titration at the mercury anode, thus avoiding the possible effect of the generator electrode on the potential of the indicator electrode when the titration is followed potentiometrically. During the electrolysis a film is formed on the mercury surface, causing fluctuations of the current; therefore the solution should be stirred vigorously. The results obtained were not sufficiently reproducible when repeated determinations were made in the same electrolyte, mainly because the quantity of precipitate formed increased during the electrolysis, which made it difficult to detect the correct equivalence point. This disadvantage led us to develop procedures for electrolysis at the platinum electrode. We first developed a method based on the addition of perchloric acid in excess to a solution of the base in the catholyte, and back-titration.

Procedure. In the electrolytic vessel put supporting electrolyte [0·1*M* sodium perchlorate in a mixture of acetic acid and acetic anhydride (1:6)], add 0·3–0·4 ml of 0·1*M* perchloric acid and 3 drops of indicator (0·1% solution of Crystal Violet in acetic acid) to the catholyte, and pass a constant current of 5–8 mA till the indicator colour change gives maximal change in absorbance. At the same time adjust the apparatus to optimal sensitivity. Then add the base to be titrated and excess of perchloric acid to the catholyte, and determine the excess of perchloric acid.

Discussion. Several determinations can be made in the same solution (we usually made 3–5). This method gives very good and reproducible results (Table I), and makes possible the determination not only of tertiary, but of secondary and primary amines, the latter two otherwise being acetylated in the supporting electrolyte when titrated directly. The method cannot, however, be applied to the determination of hydrochlorides of bases; in such cases mercury(II) acetate has to be added and during the electrolysis mercury(II) is reduced rather than the hydrogen ion, thus making the determination impossible. The main disadvantage of the method is the need to prepare and measure out the standard solution of perchloric acid, whereby the main advantage of coulometric titrations—no need to use standard solutions—is lost.

For these reasons we developed a method for the direct determination of bases in the anolyte, at a platinum electrode. The failure to determine bases directly at a platinum anode whereas they can be determined with a mercury anode can be attributed to the much lower anode potential at which the

TABLE I.—COULOMETRIC DETERMINATION OF SOME BASES IN A MIXTURE OF ACETIC ACID–ACETIC ANHYDRIDE BY THE BACK-TITRATION METHOD

Substance titrated	No. of coulom. titns.	Taken for coulom. titn., *mg*	Found by potentiom. titn., %	Found by coulom. titn., %	Average deviation, %
Potassium hydrogen phthalate	6	6·9–7·3	100·0	100·1	0·2
Sodium formate	5	2·9	99·6	99·8	0·2
Sodium benzoate	4	5·3	100·0	99·6	0·2
Sodium veronal	5	5·0	100·0	99·7	0·1
Sodium salicylate	5	5·7	99·7	100·1	0·3
Diethylamine	6	4·0–4·5	99·5	99·6	0·5
Triethylamine	5	4·2	100·0	100·0	0·2
Triethanolamine	6	4·0–4·5	99·6	99·7	0·3
Nicotinamide	6	4·8–6·6	99·6	99·8	0·1
γ-Picoline	5	5·2–5·7	99·0	98·9	0·3
Pyridine	5	3·3	96·0	95·9	0·7
Antipyrine	6	4·9–5·6	100·4	100·3	0·4
Cinchonine	5	4·8–8·4	97·2	97·4	0·4
Flagyle	5	6·2	99·9	99·5	0·2
Isonicotinic acid	5	3·5	98·6	98·7	0·6
Caffeine	6	4·0–5·0	99·7	99·9	0·2

oxidation takes place at the mercury electrode. Thus, if a more suitable anode depolarizer could be found, it would be possible, even at a platinum electrode, to generate hydrogen ions at 100% current efficiency and determine bases. We had earlier developed procedures for the determination of bases in glacial acetic acid in the presence of quinhydrone electrodes by the dead-stop method and potentiometrically at constant current.[11,12] It was of interest, therefore, to find out whether the electrolysis could be performed, without decomposition of bases, at the higher current densities customarily used in coulometric titrations. Figure 2 shows the changes in anode potential with current density. It can be seen that hydroquinone oxidation takes place at an anode potential ~0·5 V lower than that at which the acetate is oxidized. This is why in the presence of hydroquinone the hydrogen ion can be generated at 100% current efficiency. When triethylamine is added to the supporting electrolyte not containing hydroquinone, the curve remains practically unchanged. Nevertheless, the fact that anode

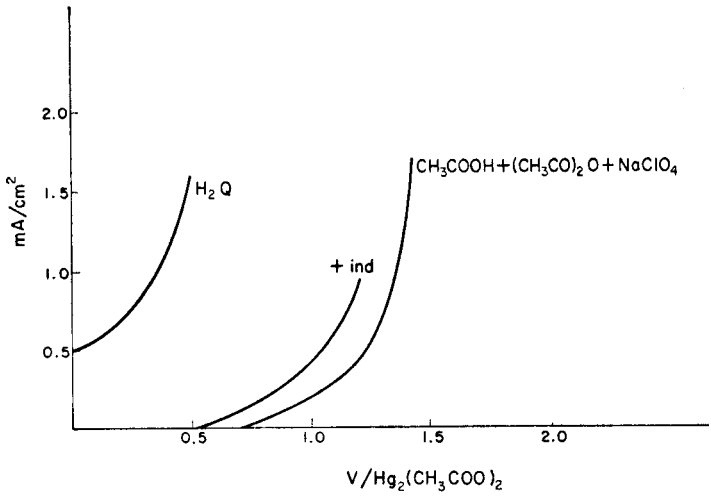

FIG. 2.—Changes in anode potential with current density.
Supporting electrolyte—20 ml of 0·1*M* $NaClO_4$ in CH_3COOH–$(CH_3CO)_2O$ (1:6). Crystal Violet (3 drops of 0·1% solution in acetic acid) added to supporting electrolyte. H_2Q—100 mg of hydroquinone added to supporting electrolyte.

TABLE II.—COULOMETRIC TITRATION OF POTASSIUM HYDROGEN PHTHALATE IN ACETIC ACID

Sample	Consecutive determination*	Time of electrolysis, *sec*	Amount found		Deviation, %
			mg	%	
I	1	428·5	6·23	99·4	−0·4
	2	427·8	6·22	99·2	−0·6
	3	429·4	6·26	99·6	−0·3
II	1	434·8	6·29	100·3	0·5
	2	435·6	6·30	100·5	0·7
	3	429·6	6·26	99·6	−0·2
				Mean 99·8₅	±0·5

* In the same electrolyte.

Sample (6·271 mg) dissolved in 0·900 ml of acetic acid. Supporting electrolyte: 20 ml of 0·1*M* $NaClO_4$ in 1:6 mixture of acetic acid and acetic anhydride with 0·1 g of hydroquinone and 3 drops of 0·1% solution of Crystal Violet in acetic acid added.

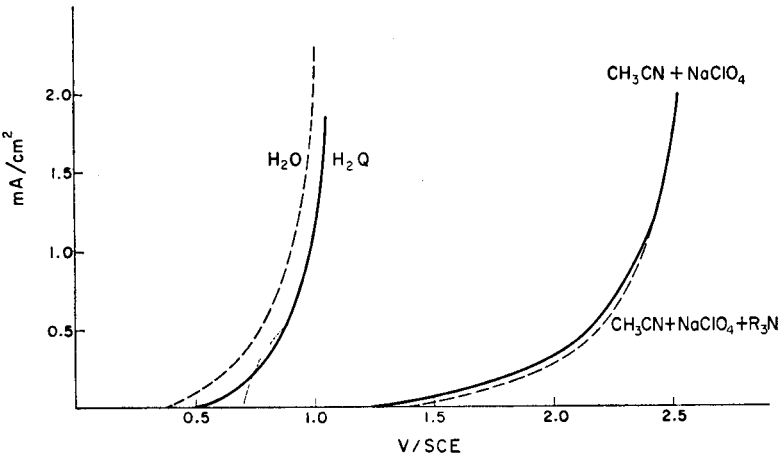

FIG. 3.—Changes in anode potential with current density in acetonitrile.
Supporting electrolyte—25 ml of 0·25*M* $NaClO_4$ in acetonitrile.
R_3N—100 mg of triethylamine added to supporting electrolyte.
H_2Q—100 mg of hydroquinone added to supporting electrolyte.
H_2O—100 mg of water added to supporting electrolyte.

potential drops on the addition of some amine-type indicators (*e.g.*, Crystal Violet, Malachite Green), and that these indicators decompose during the electrolysis, points out the possible oxidation of some amines in the solution analysed. This oxidation is, however, suppressed by the addition of hydroquinone, which itself is oxidized at a lower potential. Hydrogen ions form directly from hydroquinone, according to the equation: $C_6H_4(OH)_2 - 2e \rightarrow C_6H_4O_2 + 2H^+$. The hydrogen ions protonate the base.

On the basis of this we have developed a procedure for direct coulometric titration of bases at a platinum anode, in the presence of hydroquinone as anode depolarizer and source of hydrogen ions.

Procedure. To 25 ml of anolyte solution, consisting of 0·1*M* sodium perchlorate in acetic acid and acetic anhydride (1:6), add about 0·1 g of hydroquinone and 3 drops of Malachite Green solution, and titrate till the indicator changes from green to yellow. Then add a definite amount of

TABLE III.—COULOMETRIC DETERMINATION OF SOME BASES IN ACETONITRILE.

Substance titrated	No. of coulom. titns.	Taken, *mg*	Found by potentiom. titn., %	Found by coulom. titn., %	Average deviation %
Triethylamine	9	3·1–3·2	100·0	99·7	0·3
Monoethanolamine	6	2·5–6·0	99·3	98·3	0·5
Diethanolamine	7	3·8	99·1	99·3	0·7
Triethanolamine	8	4·2–5·2	99·6	100·0	0·7
Aniline	11	4·5–4·8	99·3	99·6	0·3
8-Hydroxyquinoline	6	2·3–2·7	99·7	99·8	1·0
γ-Picoline	6	4·8	99·0	99·1	0·6
Nicotinamide	7	3·3	99·6	100·0	0·5
p-Toluidine	10	3·1–4·2	100·0	99·9	0·3
Caffeine	7	1·7–4·3	99·7	99·1	0·4
2-Methyl-5-nitro-imidazole	4	2·6–5·4	99·3	98·4	0·2
n-Propylamine	3	1·1–2·5	99·9	99·0	0·2
Urea	4	1·7–4·2	99·4	97·8	0·3
Thiocarbamide	2	3·0	98·6	97·7	0·2

Medium was 0·1*M* $NaClO_4$, 0·05*M* hydroquinone plus 3 drops of 0·1% Malachite Green solution per 20 ml.

the base to be determined, dissolved in acetic acid, and titrate at the same current to the same colour change.

Here too several determinations can be made in the same solution; some results are given in Table II.

Coulometric determination of bases in acetonitrile

On a similar principle bases can be determined in acetonitrile, hydrogen ions being generated at 100% current efficiency in the presence of sodium perchlorate and hydroquinone. From Fig. 3 it can be seen that water and hydroquinone are oxidized before the substance analysed or the supporting electrolyte. The titration end-point was detected photometrically by means of Malachite Green, which proved to be the most suitable indicator among the 15 of different types investigated.

The procedure is identical with that described for the direct determination of bases in acetic acid. The supporting electrolytes are 3% sodium perchlorate solution in water-free acetonitrile for the anolyte, and 3% silver perchlorate solution in acetonitrile for the catholyte.

With this solvent, all types of amines can be determined individually (including urea, $pK = 14$ in water), and in certain mixtures if two indicators—Crystal Violet and Eosin— are used.

Results are presented in Table III. Potentiometric determinations are compared with coulometric titration in acetonitrile; the greatest discrepancy amounts to about 1%.

The suggested method permits the determination of primary, secondary and tertiary amines in acetonitrile, even when they are very weak bases. It makes possible the generation of hydrogen ions in acetonitrile and thus the determination of bases in that solvent, with no other solvents introduced during the titration—usually in titration of bases in acetonitrile a standard solution of perchloric acid in either acetic acid or dioxane is used; solutions of perchloric acid in acetonitrile are not used since after standing for only 10–15 min the acidity changes because of polymerization.[13]

Zusammenfassung—Arbeitsbedingungen und Ergebnisse der coulometrischen Titration von Milligrammengen einiger Basen in 0,1*M* Natriumperchlorat in einem Gemisch von Essigsäure und Acetanhydrid (1:6) werden angegeben. Die Analysen wurden sowohl mit coulometrischer Rücktitration als auch mit direkter Titration an der Platinanode ausgeführt. Die Rücktitration fand im Katholyten durch coulometrische Titration des Überschusses an zugegebener Überchlorsäure statt. Der Titrationsendpunkt wurde photometrisch mit Kristallviolett als Indikator bestimmt. Die direkte Titration der Basen wurde im selben Elektrolyten an der Anode durchgeführt wobei Hydrochinon als anodischer Depolarisator und als Protonenlieferant eingesetzt wurde, Malachitgrün als Indikator. Entsprechend können Basen bei Zusatz von Natriumperchlorat, Hydrochinon und Malachitgrün in Acetonitril bestimmt werden. Die Fehler liegen unter 1%, die Genauigkeit ist zufriedenstellend.

Résumé—On donne les conditions de travail et les résultats pour le titrage coulométrique de quantités de l'ordre du milligramme de quelques bases en perchlorate de sodium 0,1*M* dans un mélange d'acide acétique et d'anhydride acétique (1:6). Les déterminations ont été effectuées par titrage coulométrique tant en retour que direct à l'anode de platine. Le titrage en retour a été fait dans le catholyte, par titrage coulométrique de l'excès d'acide perchlorique ajouté. Le point de fin de titrage a été déterminé photométriquement avec le Cristal Violet pour indicateur. Le titrage direct de bases a été fait à l'anode de platine, dans le même électrolyte, auquel on a ajouté de l'hydroquinone comme agent dépolarisant de l'anode et comme source d'ions hydrogène, le Vert Malachite étant utilisé comme indicateur. De manière semblable, on peut déterminer les bases en acétonitrile si le perchlorate de sodium, l'hydroquinone et le Vert Malachite sont ajoutés au solvant. Les erreurs sont inférieures à 1% et la précision est satisfaisante.

REFERENCES

1. A. C. Streuli, *Anal. Chem.*, 1956, **28**, 130.
2. B. W. Mather and C. F. Anson, *Anal. Chim. Acta*, 1959, **21**, 468.
3. W. Huber, *Titrationen in nichtwässrigen Lösungsmitteln*, p. 230. Akad. Verlaggesellschaft, Frankfurt a.M., 1964.

4. G. Durand and B. Trémillon, *Bull. Soc. Chim. France*, 1963, **30,** 2867.
5. J. Vedel and B. Trémillon, *J. Electroanal. Chem.*, 1959, **1,** 241.
6. A. Schmidt and J. Noack, *Z. Anorg. Allgem. Chem.*, 1958, **296,** 263.
7. R. B. Hanselman and A. C. Streuli, *Anal. Chem.*, 1956, **28,** 916.
8. I. M. Kolthoff, M. K. Chantooni and S. Bhowmik, *ibid.*, 1967, **39,** 1627.
9. A. P. Kreshkov, L. N. Bikova and N. A. Kazaryan, *Kislotno-osnovnoe titrovanie v nevodnih rastvorah*, p. 70. Khimiya, Moscow, 1967.
10. I. Gyenes, *Titrálás nemvizes közegben*, p. 78. Müszaki könyvkiadó, Budapest, 1960.
11. V. Vajgand and T. Pastor, *J. Electroanal. Chem.*, 1964, **8,** 49.
12. *Idem, ibid.*, 1964, **8,** 40.
13. M. K. Chantooni, *Ph.D. Thesis*, University of Minnesota, 1961.

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SHORT COMMUNICATIONS

Potentiometric determination of successive stability constants of ethylenediamine complexes of several metals in dimethylsulphoxide

(*Received* 12 *December* 1968. *Accepted* 15 *January* 1969)

DIMETHYLSULPHOXIDE (DMSO) has become a popular non-aqueous solvent for various organic syntheses and kinetic studies. The solubility of organic and inorganic compounds in this solvent affords the experimentalist with increased opportunities for chemical studies not possible in the more traditional solvents.

This study was undertaken to obtain some data concerning the stabilities of several ethylenediamine–metal complexes. The results for silver, cadmium, zinc, and manganese are presented here and compared with data previously reported for studies on aqueous systems.

EXPERIMENTAL

The DMSO used in this study was treated for several days with a calcium oxide–magnesium oxide mixture and then filtered through a fine porosity sintered-glass filter. According to Butler,[1] this treatment for removal of water is generally sufficient for most electrochemical studies.

Ethylenediamine was refluxed over calcium oxide for several hours and then fractionally distilled. The middle 70% of the distillate was retained. DMSO solutions of ethylenediamine 0·10*M* in potassium perchlorate were standardized by aqueous pH titration with standard hydrochloric acid.

All solutions were prepared with an ionic strength of approximately 0·10. Silver nitrate solutions were prepared by weight from the solid and stored away from light.

Anhydrous perchlorate salts of the other metals studied (Cd, Zn, and Mn) were prepared by treatment of the hydrated salts with 2,2-dimethoxypropane.[2,3] DMSO solutions of these metals (all *ca.* 0·033*M*) were standardized by aqueous EDTA titrations.

All potential measurements were made at 25·0 ± 0·1° in a three-compartment cell. The middle compartment, separated from both the reference electrode and indicating electrode compartments by sintered glass frits, was filled with 0·1*M* potassium perchlorate in DMSO.

The reference electrode was zinc amalgam with saturated zinc perchlorate in DMSO, as recommended by McMasters *et al.*[4] The indicating electrode in silver-containing solutions was prepared by plating silver from an argentocyanide bath on to a platinum wire spiral electrode. For solutions of Zn^{2+} and Cd^{2+} ions, the respective amalgams were prepared by alternate plating of mercury and the metal on to the same platinum electrode.

Potentials were measured with a Leeds and Northrup "Students' Potentiometer".

Procedure

A known volume of metal-containing solution and the proper electrode were placed in the cell and purified nitrogen was bubbled through to remove oxygen and stir the solution.[5] The potential generally became stable after a period of 15–60 min. Portions of 1·0*M* ethylenediamine solution were then delivered into the cell by means of a 10-ml burette or, in some experiments, pure dry ethylenediamine was delivered from a weighed syringe. The solution was then mixed thoroughly by bubbling nitrogen and allowed to stand quiescently until the potential no longer drifted. Generally, equilibrium was attained within the mixing time. At least three replicate titrations for each metal were performed in this way for silver, zinc, and cadmium. Replicate measurements on all systems studied usually agreed within 3 or 4 mV.

Solutions containing both manganese and silver were titrated in the same way, with a silver indicator electrode.

MATHEMATICAL TREATMENT

The measured potential difference, (ΔE), between cell potentials when complexing agent is present and when it is absent, is given by the well known equation:

$$\exp\left[-\frac{nF(\Delta E)}{RT}\right] = 1 + \sum_{i=1}^{N} \beta_i [\mathrm{L}]^i \qquad (1)$$

where n = number of electrons involved in the potential-determining couple, N = total number of possible complexes, [L] = free ligand concentration (M) and β_i = overall stability constant for the ith complex ion.

The formal (total) ligand concentration, F_L, is given by:

$$F_L = [L] + C_M\bar{n} \tag{2}$$

where C_M = formal metal ion concentration, and

$$\bar{n} = \frac{\sum_{i=1}^{N} i\beta_i[L]^i}{1 + \sum_{i=1}^{N} \beta_i[L]^i} \tag{3}$$

The experimental values of ΔE and F_L, when plotted, represent a titration curve.

For the case of mixed manganese and silver solutions, the ligand conservation equation becomes:

$$F_L = [L] + C_{Ag}\bar{n}_{Ag} + C_{Mn}\bar{n}_{Mn} \tag{4}$$

Since the presence of Mn^{2+} does not interfere with the potential-determining couple, Ag/Ag^+, measurement of ΔE in the mixed solution, along with the previously determined values of the silver-ethylenediamine formation constants, allows immediate calculation of [L] and $\bar{n}_{Ag}$ for each point. Use of equation (4) and the knowledge of [L] and $\bar{n}_{Ag}$ allows the determination of $\bar{n}_{Mn}$. From the plot of $\bar{n}_{Mn}$ *vs.* log [L], the values of [L] corresponding to $\bar{n}_{Mn} = \frac{1}{2}$, $\frac{3}{2}$, and $\frac{5}{2}$ were determined and equation (3) was applied. The resulting 3 equations in 3 unknowns, (β_1, β_2, and β_3 for Mn) were solved simultaneously. The effect of dilution was properly included in the data reduction process.

RESULTS

To apply the foregoing equations, the free ligand concentration, [L], must be known for each data point. Without knowledge of the stability constants, [L] cannot be computed except under the conditions where $F_L/C_M > N$, *i.e.*, after the end point of the titration. Several algorithms for computer calculation of pre-end-point values of [L] and β's simultaneously, were tried without success for the case where $N = 3$. The $N = 2$ case, (Ag), was successfully handled by an iterative computer calculation. Refinement of the calculations for silver required consideration of the complex Ag_2L^{2+}. The data were best fitted for $\log_{10} K_f = 5{\cdot}8$. A trial and error method was utilized to obtain this value. The $N = 3$ cases, Zn and Cd, were treated by trial and error methods to obtain reasonable values of β_1 and β_2 which adequately reproduced the lower section of the experimental titration curves. β_3, of course, is the most accurately computable formation constant and is obtained from post-end-point data.

The values of the formation constants that best fitted the experimental data are given in Table I, along with estimates of error. Also included are the corresponding formation constants determined in water.[6]

Figure 1 shows the experimental titration curve for the silver–ethylenediamine system and the lines constructed from the best values obtainable for the formation constants. It is apparent that the fit is better when the influence of the species Ag_2en^{2+} is included. The zinc–ethylenediamine and cadmium–ethylenediamine systems gave an even better overall fit.

Figure 2, $\bar{n}_{Mn}$ *vs.* log [L], shows the experimental points from the mixed Mn^{2+}–Ag^+ solution experiments and the line constructed from the values of manganese formation constants given in Table I.

TABLE I.—LOGARITHMS OF ETHYLENEDIAMINE–METAL OVERALL STABILITY CONSTANTS IN DMSO AND WATER[6] AT 25°C AND IONIC STRENGTH 0·1*M*

Metal	DMSO			H_2O†		
	β_1	β_2	β_3	β_1	β_2	β_3
Ag*	6·27 ± 0·10	9·54 ± 0·04	—	4·7	7·70	—
Zn	7·18 ± 0·10	13·85 ± 0·06	18·70 ± 0·04	5·7	10·4	13·1
Cd	7·0 ± 0·1	13·0 ± 0·1	17·63 ± 0·08	5·5	10·1	12·3
Mn	3·7 ± 0·2	6·9 ± 0·2	10·1 ± 0·2	2·77	4·8	5·7

† Weighted average of values given in reference 6 are listed in the last three columns.

* For the silver–ethylenediamine system, the complex Ag_2en^{2+} was included in the calculations and the logarithm of the formation constant in DMSO is 5·8. In water, $\log_{10} K_f$ for $Ag_2en^{2+} = 6{\cdot}5$ as given by Schwarzenbach *et al.*[7]

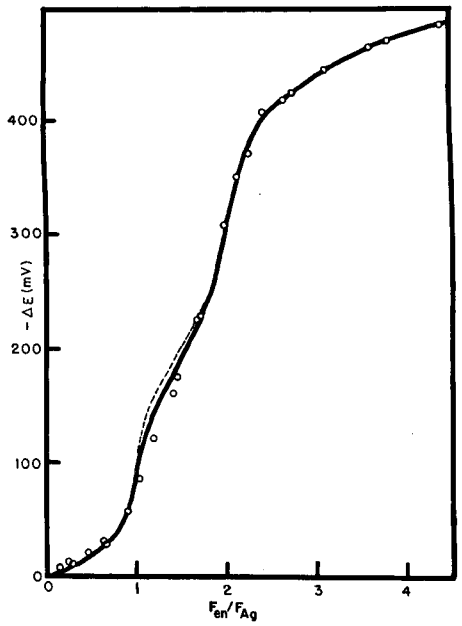

FIG. 1.—Titration curve of silver ion in DMSO with ethylenediamine. Solid line constructed from formation constants listed in Table I along with log K_1 for Ag_2en^{2+} = 5·8. Broken line constructed using log β_1 = 6·4 and ignoring the presence of Ag_2en^{2+}. The open circles represent mean values of duplicate experimental points.

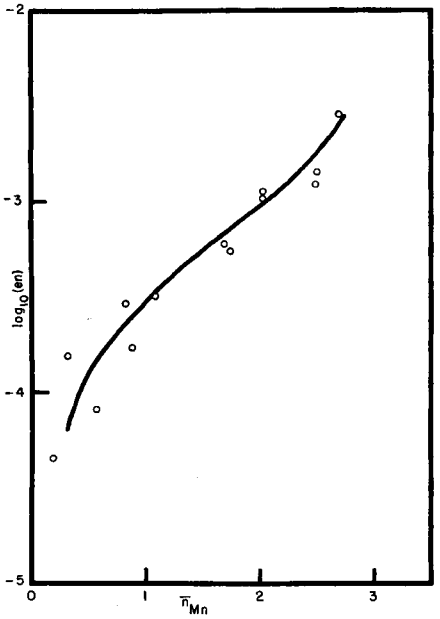

FIG. 2.—Formation curve for the manganese(II)–ethylenediamine system. Solid curve calculated from values of formation constants given in Table I. Open circles represent experimental points.

DISCUSSION

Presumably the energy of the metal–nitrogen bonds formed in the complex ions will be nearly independent of the surrounding solvent. However, the solvation energy of the metal ions alone will be different and this is probably the main contribution to the obviously larger numerical values for the corresponding formation constants in DMSO when compared to water. Also, the solvation energy of ethylenediamine alone is probably different in the two solvents being considered here. For both the metals and ligand, one would expect lower solvation energy in DMSO than in water owing to its lower dielectric constant.

Quantitative correlation of the differences in formation constants *via* the Born expression for free energies of solvation[8] depends on knowledge of effective radii of the solvated ions. Unfortunately such data are sparse. Also, zinc and cadmium as solvated ions are generally 4-co-ordinate (tetrahedral), but their co-ordination spheres are expanded in the presence of excess of ethylenediamine to 6-co-ordinate (octahedral). Considering these factors, it is not surprising that a good quantitative correlation of the differences in formation constants could not be obtained.

CONCLUSIONS

The complex ions formed from ethylenediamine and the metals studied in this work are essentially the same as those formed in water, with apparent stability of the complexes greater in DMSO than in water. Qualitatively, the greater apparent stability can be attributed to a lower solvation energy of the unchelated metal ion in DMSO, but quantitative correlation is difficult.

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Summary—The stability constants of the complexes of silver(I), zinc(II), cadmium(II) and manganese(II) with ethylenediamine in dimethylsulphoxide at 25° and ionic strength 0·1*M* have been determined potentiometrically. The stability constants are consistently larger in DMSO than in water as expected from the difference in dielectric constant for the two solvents.

Zusammenfassung—Die Stabilitätskonstanten der Komplexe von Silber(I), Zink(II), Cadmium(II) und Mangan(II) mit Äthylendiamin in Dimethylsulfoxid bei 25° und der Ionenstärke 0,1*M* wurden potentiometrisch bestimmt. Die Stabilitätskonstanten sind in Dimethylsulfoxid durchweg größer als in Wasser, wie nach dem Unterschied in den Dielektrizitätskonstanten der beiden Lösungsmittel zu erwarten war.

Résumé—On a déterminé potentiométriquement les constantes de stabilité des complexes d'argent(I), zinc(II), cadmium(II) et manganèse(II) avec l'éthylènediamine en diméthylsulfoxyde, à 25° et pour une force ionique 0,1*M*. Les constantes de stabilité sont essentiellement plus grandes en DMSO que dans l'eau, comme attendu de la différence entre les constantes diélectriques des deux solvants.

REFERENCES

1. J. N. Butler, *J. Electroanal. Chem.*, 1967, **14**, 89.
2. K. Starke, *J. Inorg. Nucl. Chem.*, 1959, **11**, 77.

* Present address: Atlantic Richfield Hanford Company, Box 250, Richland, Washington 99352, U.S.A.

3. D. W. Meek, R. R. Drago and T. S. Piper, *Inorg. Chem.*, 1962, **1**, 285.
4. D. L. McMaster, R. B Dunlap, J R. Kuempel, L. W. Kreider and T. R. Sherer, *Anal. Chem.*, 1967, **39**, 103.
5. K. H. Pool, *J. Polarographic Soc.*, 1967, **13**, 23.
6. L. G. Sillén and A. E. Martell, *Stability Constants of Metal-Ion Complexes*, Chem. Soc. Special Publication No. 17, London, 1964.
7. G. Schwarzenbach, H. Ackerman, B. Maissen and G. Anderegg, *Helv. Chim. Acta*, 1952, **35**, 2337.
8. M. Born, *Physik. Z.*, 1920, **1**, 45.

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Determination of cerium in minerals with sulphanilic acid

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THE determination of cerium with sulphanilic acid[1] is lacking in sensitivity. In a previous procedure using this reagent, the instability of the coloured complex was so significant that the absorbance was recommended to be read exactly 3 min after colour development had started, and even then the absorbance changed continuously.

The parameters affecting precision of the previous methods have been investigated.[2] The critical parameters were acid concentration both at the time of cerium oxidation and colour development, and temperature at the time of addition of the sulphanilic acid reagent.

The optimum sulphuric acid concentration range for the oxidation of cerium(III) to cerium(IV) was 2–6%.[3–6] The stability of the colour developed was greatest at 20% sulphuric acid concentration. The temperature was critical at the time of addition of sulphanilic acid and the absorbance varied inversely with temperature. If these conditions are controlled, the absorbance is stable and repeatable for the time interval between 15 and 60 min after addition of the sulphanilic acid reagent. For optimum precision it would be desirable to run a standard with each batch of samples.

EXPERIMENTAL

Reagents

Sulphanilic acid solution, 1%.

Silver nitrate solution, 0·25%.

Standard cerium((IV) solution (1 mg/ml). Prepared in 5% sulphuric acid, from ceric ammonium sulphate dihydrate.

Standard cerium(III) solution (1 mg/ml). Prepared by dissolving 1 g of cerium metal in 200 ml of 2·5% sulphuric acid and diluting to 1 litre with water.

Lanthanum co-precipitating reagent. Prepared by dissolving lanthanum oxide in sulphuric acid and diluting to a lanthanum concentration of 5 mg/ml.

Oxalate-nitric acid wash solution. Prepared by dissolving 30 g of ammonium oxalate in 960 ml of water and adding 40 ml of nitric acid.

Procedure

Dissolve the mineral sample in 3 ml of sulphuric acid and sufficient nitric acid to dissolve the rare earth minerals. Heat the solution to fuming. Add 25 ml of water and filter. Dilute the filtrate to to approximately 50 ml with water, add 5 drops of silver nitrate solution and boil. Add 0·5–1·0 g of ammonium persulphate and continue boiling for 3 min. Cool and transfer to a 100-ml volumetric flask. Add 50 ml of 50% sulphuric acid and cool the solution to room temperature and dilute to volume with water. Take an aliquot (up to 20 ml) from this solution and transfer it to a dry 25-ml volumetric flask. Adjust the volume to 20 ml with 25% sulphuric acid if necessary, and place in a water bath at 15–20°. Add 5 ml of sulphanilic acid reagent and make up to volume with water. Determine the absorbance at 540 nm, in 10- or 50-nm cells, during the next 15–60 min.

The only elements normally found in minerals that interfere in this determination are manganese and chromium. In the presence of other than trace amounts of these elements cerium must first be

separated. The oxalate precipitation in the presence of lanthanum as a gathering agent is used, as follows. After dissolution of the minerals, evaporate the solution to fumes of sulphuric acid and then adjust the volume of 60 ml with water and filter. Adjust the volume to 80 ml with water and add 10 ml of lanthanum solution. Adjust the pH to 1·7–1·8 with ammonium hydroxide or nitric acid. Add 4 ml of nitric acid and adjust the volume to 100 ml with water. Boil, add 5 g of ammonium oxalate and continue boiling for 5 min. Cool and let stand for 30 min with occasional stirring. Filter off and wash with 50–75 ml of oxalate-nitric acid wash solution. Return the precipitate and filter paper to the original beaker. Add 20 ml of nitric acid and 3 ml of sulphuric acid. Evaporate to charring of the paper and successively add hydrogen peroxide and nitric acid until there is no further evidence of charring. Add nitric acid and evaporate to fumes of sulphur trioxide. Cool, then oxidize cerium-(III) as above. If separation from manganese is not complete, the permanganate colour will appear and a second separation with oxalate can be performed.

RESULTS AND DISCUSSION

Samples containing 5 mg of cerium(III) were oxidized by the silver nitrate and ammonium persulphate procedure over a range of sulphuric acid concentrations. When the colour was developed with the sulphanilic acid reagent, oxidation was found to be complete with addition of 0·6–3·6 ml of sulphuric acid in a 50-ml volume as indicated in Table I.

Table II shows the effect of the temperature of the solution at the time of addition of the sulphanilic acid reagent. Table III indicates the precision of standard curves; it appears to be adequate for determination of cerium in minerals in the range 0·1–20%.

TABLE I.—OXIDATION OF Ce^{3+} TO Ce^{4+}

H_2SO_4, *ml*	Absorbance at 540 *nm*	Ce^{4+} recovered, %
0·1	0·238	49
0·6	0·476	99
1·1	0·482	101
1·6	0·479	100
2·1	0·479	100
2·6	0·479	100
3·6	0·477	100
5·6	0·397	82

TABLE II.—EFFECT OF TEMPERATURE WHEN SULPHANILIC ACID IS ADDED, ON ABSORBANCE

Temp., °C	Time, *min*									
	5	10	15	20	30	45	60	75	90	150
30	0·938	0·926	0·932	0·934	0·936	0·936	0·936	0·924	0·922	0·898
15	1·003	0·989	0·995	0·998	0·998	1·000	1·003	1·001	0·989	0·953
0	1·138	1·110	1·098	1·098	1·104	1·107	1·110	1·108	1·090	1·043

TABLE III.—STANDARD CURVES FOR Ce

Ce, *mg*	Abs. at 540 *nm*		Abs. per 1·0 *mg* of Ce	
	10-mm cell	50-mm cell	10-mm cell	50-mm cell
0·0	0·000	0·000	—	—
0·5	—	0·221	—	0·442
1·0	—	0·466	—	0·466
2·0	0·190	0·936	0·0948	0·468
5·0	0·481	—	0·0962	—
10·0	0·959	—	0·0959	—

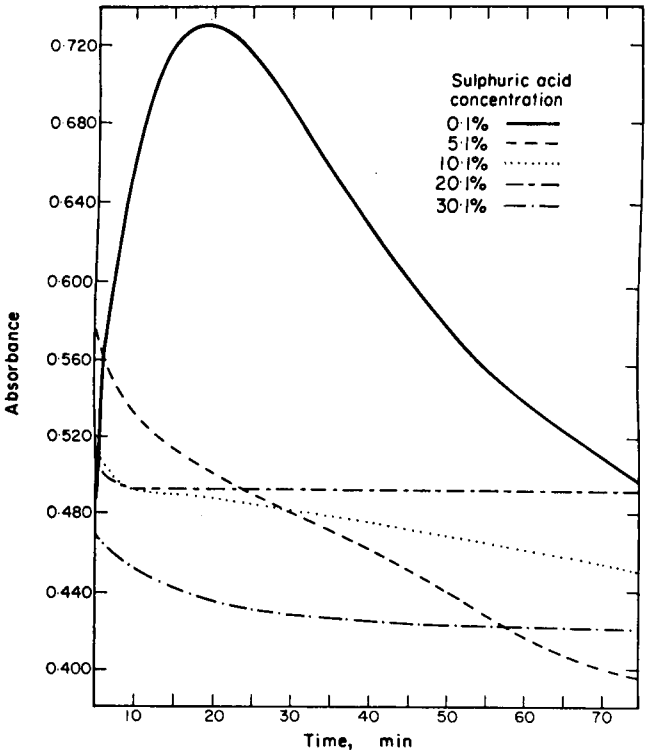

FIG. 1.—Stability of the colour produced by sulphanilic acid oxidation.

Figure 1 shows the stability that can be achieved when colour development takes place in 20% sulphuric acid.

Recovery of 15–30 mg of cerium from synthetic samples that required prior separation by the oxalate precipitation procedure was 96–99%. To obtain reasonably complete recovery of cerium in samples with low concentrations, it is most desirable to use lanthanum as a co-precipitant.

TABLE IV.—ANALYSIS OF MINERALS

Samples	Ce,	%
A	2·46	2·49
B	0·99*	1·08
C	28·0	27·9
D	5·30	4·98*
E	2·86	2·92

* Pyrosulphate fusion.

Table IV illustrates the results which may be obtained with mineral samples containing 1–30% cerium. These analyses were performed on duplicate portions of powders. The results from powder samples dissolved in sulphuric acid–nitric acid agreed reasonably well with those for powder samples dissolved by pyrosulphate fusion.

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Summary—Cerium can be determined colorimetrically in minerals with sulphanilic acid. Cerium(III) ions are oxidized with silver(II) in 1–7% sulphuric acid. Sulphanilic acid is oxidized by cerium(IV) ions in 20% sulphuric acid. The absorbance is determined at 540 nm. In the presence of maganese or chromium, cerium can be separated by precipitation as the oxalate. Lanthanum can be used as a gathering agent if necessary.

Zusammenfassung—Cer in Mineralien kann kolorimetrisch mit Sulfanilsäure bestimmt werden. Cer(III)-Ionen werden in 1–7% Schwefelsäure mit zweiwertigem Silber oxidiert; die Sulfanilsäure wird in 20% Schwefelsäure von Cer(IV)-Ionen oxidiert. Die Extinktion wird bei 540 nm gemessen. In Gegenwart von Mangan oder Chrom kann Cer durch Fällung als Oxalt abgetrennt werden. Notfalls kann man Lanthan als Träger verwenden.

Résumé—On peut doser le cérium colorimétriquement dans les minéraux au moyen d'acide sulfanilique. Les ions cérium(III) sont oxydés par l'argent(II) en acide sulfurique à 7%. L'acide sulfanilique est oxydé par les ions cérium(IV) en acide sulfurique à 20%. On détermine l'absorption à 540 nm. En la présence de manganèse ou de chrome, on peut séparer le cérium par précipitation à l'état d'oxalate. On peut si nécessaire utiliser le lanthane comme agent de rassemblement.

REFERENCES

1. P. L. Sarma and L. H. Dieter, *Talanta*, 1966, **13**, 347.
2. G. Somiclevamma and M. S. Sarma, *ibid.*, 1967, **14**, 861.
3. H. Onishi and C. V. Banks, *Anal. Chem.*, 1963, **35**, 1887.
4. I. s, E. Schulek and M. Rózsahegyi, *Talanta*, 1963, **10**, 445.
5. S. F. Marsh, W. J. Maeck, G. L. Booman and J. E. Rein, *Anal. Chem.*, 1962, **34**, 1406.
6. L. A. Knecht, *Anal. Chim. Acta*, 1967, **39**, 349.
7. N. Jordanov, A. Antonova and C. Daiev, *Talanta*, 1966, **13**, 1459.

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Determination of uranium in rocks by instrumental activation-analysis using epithermal neutrons

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THE uranium content of geological material can be determined in various ways by neutron-activation methods. A number of workers have based the determination on the isolation of a fission product, *e.g.*, ^{140}Ba,[1–4] ^{103}Ru[5] or ^{133}I.[6] Other investigators have used delayed neutron counting.[7,8] Radiochemical methods utilizing either 23·5 min ^{239}U[9,10] or 2·3 d ^{239}Np[11–13] have also been described.

The determination of uranium in rocks by thermal neutron-activation and γ-spectrometry, without any chemical separation step, is generally impossible, owing to the low uranium content normally present in rocks (of the order of 1 ppm). Activation with epithermal neutrons, however, enhances the formation of ^{239}U compared to other radionuclides, owing to the high resonance activation integral of ^{238}U. The present work demonstrates the instrumental determination of uranium in rocks with concentrations in the 1 ppm region, by resonance neutron activation, followed by γ-spectrometry with a thin sodium iodide crystal. Five U.S. Geological Survey standard rocks, with uranium contents in the range 0·5–2·5 ppm, were selected as test samples for this study. In addition, two iron ore samples having uranium contents of several hundred ppm were included.

Resonance neutron activation

This technique can be favourably employed if the ratio of resonance integral to thermal neutron activation cross-section for the nuclide in question is high compared with the corresponding ratio for nuclides giving rise to major interfering activities in the sample.

Applications of instrumental resonance neutron activation have been demonstrated by Borg *et al.*[14] for the determination of manganese in biological material, and by Brune and Jirlow,[15] who determined molybdenum in steel. No applications of this technique for instrumental analyses of geological material have appeared in the literature so far. Turkowsky *et al.*,[10] in their radiochemical method for uranium, used activation with epithermal neutrons to reduce the total induced activity in their samples, in order to facilitate the handling procedure.

The feasibility of epithermal activation has been discussed by Brune and Jirlow,[15] who have defined an "advantage" factor expressed as the ratio $(R_{Cd})_d/(R_{Cd})_D$, where R_{Cd} means the cadmium ratio, and d and D denote the interfering nuclide and the nuclide under investigation, respectively. If the determination of uranium in rocks is based on 23·5 min ^{239}U, the most important interfering nuclides will be 2·3 min ^{28}Al, 2·58 hr ^{56}Mn, and 15·0 hr ^{24}Na. In the present work, where the irradiations were carried out in a neutron flux with an R_{Cd} of 2·5 for Au, the advantage factors for U, compared to Al, Mn and Na, were found to be 26, 19, and 33 respectively, the following formula[19] being used for the calculation of the cadmium ratio:

$$R_{Cd} = 1 + \frac{\sigma_0}{\sigma_0^{Au}} \cdot \frac{0{\cdot}44\sigma_0^{Au} + I^{Au}}{0{\cdot}44\sigma_0 + I} (R_{Cd}^{Au} - 1)$$

where σ_0 means the thermal neutron activation cross-section, and I the resonance activation integral excluding the $1/v$ contribution.

EXPERIMENTAL

Apparatus

A 6 × 76 mm NaI(Tl) detector, connected to a TMC 400-channel pulse-height analyser was used for the analyses. Preliminary experiments showed this system to be superior to an alternative system based on a 2 ml Ge(Li) detector.

Preparation of samples and standards

Rock samples of about 30 mg were sealed in 10 × 10 mm polyethylene bags. Before irradiation, each bag was wrapped in aluminium foil. Standards were prepared by carefully evaporating aliquots of a dilute uranium solution on small polyethylene sheets. After drying, the sheets were transferred to polyethylene bags and treated in the same manner as the samples.

For each irradiation, 4–6 samples and 2 standards were placed in a 1-mm thick cylindrical cadmium box, of 14 mm internal diameter and 10 mm internal height.

Experimental procedure

The cadmium box containing samples and standards was irradiated for 1 min in a pneumatic tube system of the reactor R2 (Studsvik, Sweden), in a position where the thermal flux was about 2×10^{11} n/mm²/sec and R_{Cd}^{Au} was 2·5.

After 5 min the samples were removed from the cadmium box, and the aluminium-foil was discarded. The counting was started 15 min after the end of the irradiation, and counting times of 1–4 min were used. The statistical counting errors were <3% in all measurements. The calculation of uranium content was based on the 74 keV peak of ^{239}U.

RESULTS AND DISCUSSION

Results for the uranium content in the various rocks studied are given in Table I. The results are compared with those of other workers using neutron-activation methods,[5,9,10,13,16] and the agreement is quite satisfactory. In Table II the results for the iron ore samples are compared with results for the same samples obtained by X-ray fluorescence spectrometry[17] and by pile neutron activation analysis based on measurement of the 229 keV γ-ray of ^{239}Np with a 23-ml Ge(Li) detector.

The precision of the present method appears to be about 5%. The sensitivity of the method, which is mainly dependent on the amounts of ^{56}Mn and ^{24}Na induced in the samples, is about 0·1 ppm. This should facilitate uranium determination in most silicate rocks.

TABLE I.—URANIUM CONTENT OF SOME STANDARD ROCKS

Standard rock	Present work		Previous neutron-activation values, *ppm*
	Single values, *ppm*	Mean value, *ppm*	
Diabase W-1	0·60, 0·57, 0·65	0·61	0·65,[5] 0·54[9] 0·59,[10] 0·55[18]
Andesite AGV-1	1·99, 1·95, 1·85	1·93	2·17[16]
Basalt BCR-1	1·96, 2·02, 1·84	1·94	1·81[16]
Granite G-2	2·14, 2·07, 1·96	2·06	2·16[16]
Granodiorite GSP-1	2·05, 2·02, 2·38 2·02, 2·38, 2·02	2·15	

Neutron shielding effects constitute a possible source of systematic error, notably in the energy region 5–200 eV, where the excitation function of ^{238}U possesses several large resonances. As none of the major components of the rock samples show appreciable resonance absorption in this energy region, the most important effects should be associated with self-shielding in the uranium. An upper limit for this self-shielding can be estimated by assuming that the cross-section for all neutrons contributing to the activation process is equal to the cross-section value at the top of the highest

TABLE II.—URANIUM CONTENT OF TWO IRON ORE SAMPLES

Sample	Epithermal activation, *ppm*	Pile neutron activation + Ge(Li), *ppm*	X-ray fluorescence, *ppm*
1	519, 514, 504 mean 512	508	510
2	288, 281, 290 mean 286	309	297

resonance, which is about 7000 barn.[18] By the method described by Høgdahl,[19] the upper limit was calculated to be less than 1% in a 30-mg rock sample even for a uranium concentration of 500 ppm. This conclusion is supported by the good agreement obtained for the iron ore samples by different techniques.

Another possible source of interference is represented by radionuclides having γ-ray energies close to the 74 keV γ-ray of ^{239}U. Consideration of the various elements in terms of their abundance in rocks, their resonance activation properties, and the γ-spectra of their isotopes formed by neutron capture, indicated 47 hr ^{153}Sm to be the only significant interference possibility. The level of this interference is negligible, however, if the samarium content is less than 200 times the uranium content, which is usually the case in silicate rocks.

The present method should be superior in speed and comparable in precision and accuracy with the radiochemical neutron-activation methods previously published for uranium in rocks. The only neutron-activation technique capable of attaining the same rapidity is probably delayed neutron counting. The major drawback of that technique, and of any technique associated with the fission of ^{235}U, is the interference introduced by the fast neutron fission of ^{232}Th. This interference becomes important in the analysis of rocks, where the thorium content is in many cases several times higher than that of uranium.

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Summary—A rapid non-destructive neutron-activation method for the determination of uranium in rocks is described. The method is based on activation with epithermal neutrons and subsequent measurement of the 74 keV γ-ray of ^{239}U. Results given for some standard rocks are in good agreement with literature data. The precision of the method is about 5% and the limit of detection is of the order of 0·1 ppm in silicate rocks.

Zusammenfassung—Ein schnelles zerstörungsfreies Neutronenaktivierungsverfahren zur Bestimmung von Uran in Gesteinen wird beschrieben. Es beruht auf Aktivierung mit epithermischen Neutronen und anschließender Messung der 74 keV-Gammastrahlung von ^{239}U. Die für einige Standardgesteine angegebenen Ergebnisse stimmen gut mit Literaturwerten überein. Die Genauigkeit des Verfahrens beträgt etwa 5%, die Nachweisgrenze in Silikatgesteinen liegt in der Größenordnung von 0,1 ppm.

Résumé—On décrit une méthode rapide et non destructive par activation de neutrons pour la détermination de l'uranium dans les roches. La méthode est basée sur l'activation par neutrons épithermiques suivie de la mesure de la raie γ 74 keV de ^{239}U. Les résultats donnés pour quelques roches étalons sont en bon accord avec les données de la littérature. La précision de la méthode est d'environ 5% et la limite de détection est de l'ordre de 0,1 ppm dans des roches aux silicates.

REFERENCES

1. A. A. Smales, *Analyst*, 1952, **77**, 778.
2. S. F. Singer, *U.S. At. Energy Comm. Rept., ORNL-76-52*, 1952.
3. C. Fischer, *U.S. At. Energy Comm. Rept., CEA-523*, 1956.
4. H. Wakita, H. Nagasawa, S. Uyeda and H. Kuno, *Earth Plan. Sci. Letters*, 1967, **2**, 377.
5. G. L. Bate and J. R. Huizenga, *Geochim. Cosmochim. Acta*, 1963, **27**, 345.
6. G. G. Goles and E. Anders, *ibid.*, 1962, **26**, 723.
7. F. F. Dyer, J. F. Emery and G. W. Leddicotte, *U.S. At. Energy Comm. Rept., ORNL-3342*, 1962.
8. S. Amiel and J. Gilat, *Geochim. Cosmochim. Acta*, 1967, **31**, 1499.
9. M. S. Das, *U.S. At. Energy Comm. Rept., TID-18204*, 1962.
10. C. Turkowsky, H. Stärk and H. J. Born, *Radiochim. Acta*, 1967, **8**, 27.
11. H. A. Mahlman and G. W. Leddicotte, *Anal. Chem.*, 1955, **27**, 823.
12. H. Hamaguchi, G. W. Reed and A. Turkevich, *Geochim. Cosmochim. Acta*, 1957, **12**, 337.
13. J. W. Morgan and J. F. Lovering, *Anal. Chim. Acta*, 1963, **28**, 405.
14. D. C. Borg, R. E. Segel, P. Kienle and L. Campbell, *Int. J. Appl. Radiation Isotopes*, 1961, **11**, 10.
15. D. Brune and K. Jirlow, *Nukleonik*, 1964, **6**, 242.
16. J. W. Morgan and K. S. Heier, *Earth Plan. Sci. Letters*, 1966, **1**, 158.
17. A. Follo, *private communication.*
18. D. J. Hughes and R. B. Schwartz, *U.S. At. Energy Comm. Rept., BNL-325*, 1958.
19. O. T. Høgdahl, *Proc. IAEA Conf. Radiochemical Methods of Analysis*, Vienna, 1965, p. 23.

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Spectrophotometric determination of palladium in titanium-base alloys, with dimethylglyoxime

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IN RECENT years, 0·1–0·2% of palladium has been added to naturally corrosion-resistant titanium alloys in order to increase resistance to stress-corrosion cracking.[1] Environments in which stress-corrosion cracking occur include aqueous chloride, bromide and iodide solutions, sodium chloride deposited on a surface at 300–450°, the H–N–O system (including fuming nitric acid and nitrogen tetroxide) and the methanol–halide–water system.

The Concorde and the Boeing SST jet engines and airframes will use large amounts of titanium alloys, *e.g.*, Ti-6Al-4V specially heat-treated for maximum strength. I. R. Lane at the Naval Ship Research & Development Center, Annapolis, Md., has done much testing of titanium alloys primarily as hull materials for deep submersibles. Candidate alloys include Ti-6Al-2Nb-1Ta-1Mo, Ti-6Al-4V (extra low interstitials) and Ti-7Al-2·5Mo. The alloy Ti-8Al-2Nb-1Ta was found to be metallurgically unstable and the stable but lower strength Ti-7Al-2Nb-1Ta proved to be susceptible to crack growth accelerated by sea-water. In both aerospace and hydrospace applications, it is expected that the high-strength, low-weight titanium alloys may require palladium additions for increased stress-corrosion resistance. Titanium and its alloys are extensively used for equipment such as reactors and heat-exchangers exposed to chloride, chlorine and sulphuric acid. Desalination equipment is expected to use large amounts of titanium alloys in the future.

These developments made it desirable to survey the many analytical methods for palladium to find one suited to this application and rapid, highly selective, convenient and reproducible. Dimethylglyoxime has been used for many years for the gravimetric determination of palladium(II) by precipitation from dilute mineral acid solution,[2] and also for removal of palladium(II) by solvent extraction into chloroform.[3]

Many colorimetric reagents of high sensitivity for palladium have been reported; Beamish[4] lists about 50 in the literature up to 1962 and others have appeared since. Little use seems to have been made of dimethylglyoxime for the colorimetric determination of palladium, probably because of the low sensitivity reported.[5] Other compounds having the reactive oxime group which have been used for determining palladium photometrically include: β-furfuraldoxime,[6] glyoxime, [7]methylglyoxime[8] salicylaldoxime,[8] α-furildioxime,[9] 4-methyl-1,2-cyclohexanedionedioxime,[10] 2,2′-dipyridyl ketone oxime,[11] phenyl 2-pyridyl ketone oxime,[12] and 2-pyridinealdoxime.[13] However, few if any can match the specificity, speed, convenience and availability of dimethylglyoxime for the colorimetric determination of palladium in titanium alloys which may contain aluminium, chromium, cobalt, copper, iron, manganese, molybdenum, niobium, tantalum, tin, vanadium and zirconium. Nielsch[3] has reported on this determination but without investigation of possible interferences or of its technical application.

Dimethylglyoxime has the advantages that the water-insoluble yellow palladium complex forms readily in chloride solutions, is easily extracted by chloroform and that both the complex in chloroform and the reagent in ethanol are stable.

EXPERIMENTAL

Reagents

Standard palladium solution. Dissolve 100 mg of palladium metal in a few ml of *aqua regia*, then dilute to 100 ml with 0·6*M* hydrochloric acid. Dilute further with 0·3*M* hydrochloric acid to obtain a standard solution containing 10 μg of palladium(II) per ml.

Dimethylglyoxime. A 1% solution in 95% ethanol.

Citric acid. A 50% aqueous solution of the monohydrate.

Potassium chloride buffer, 0·05*M* KCl–0·06*M* HCl. Dissolve 3·7 g of potassium chloride in water, add 5 ml of hydrochloric acid and dilute to 1 litre with water.

Potassium nitrate buffer, 0·25*M* KNO_3–0·25*M* HNO_3. Dissolve 25·3 g of potassium nitrate in water, add 16·7 ml of nitric acid and dilute to 1 litre with water.

Procedure

Weigh a sample of the alloy (0·4–1·0 g for 0·1–0·2% palladium content) into a 100-ml Teflon beaker. Cover the beaker and add 20–40 ml of 6*M* hydrochloric acid and 1–2 ml of fluoboric acid. Alternatively, 1·8*M* sulphuric acid may be used instead of 6*M* hydrochloric acid. Let stand or warm slightly until the reaction ceases. Add nitric acid dropwise (10–20 drops) and warm until the titanium

is oxidized and the solution is clear. The colour of the solution changes from deep purple to yellow, or red-brown if much palladium is present. Transfer to a 100-ml volumetric flask and dilute to the mark, keeping the hydrochloric acid concentration about 2·4*M*.

Transfer a sample aliquot, containing preferably 5–350 μg of palladium, to a 60-ml separatory funnel containing 1 ml of 50% citric acid solution. Add about 10 ml of chloride or nitrate buffer solution, dilute to 20 ml with water and mix. Carry a reagent blank through the procedure at the same time. Add 2 ml of 1% dimethylglyoxime solution and mix. Allow a few minutes for the complex to form; during this time, add about 0·5 g of sodium sulphate to each of the 25-ml volumetric flasks which will receive the chloroform extracts.

Shake the sample solution with 7–8 ml of chloroform for 30–60 sec, carefully release the pressure by loosening the stopper, and drain the extract into the receiving flask. Repeat the extraction twice with similar portions of chloroform and combine the extracts. Dilute to the mark with chloroform and mix. Any water droplets will be absorbed by the sodium sulphate. Measure the absorbance of the complex at 380 nm in a 50-mm cell with chloroform as reference. Prepare a calibration curve.

RESULTS AND DISCUSSION

Absorption characteristics

The chloroform solution of the complex has an absorption maximum at about 380 nm where there is negligible absorption by the reagent blank (Fig. 1). Beer's law is obeyed at least up to 12 ppm of palladium; the molar absorptivity at 380 nm is 170 l. $mol^{-1}.mm^{-1}$.

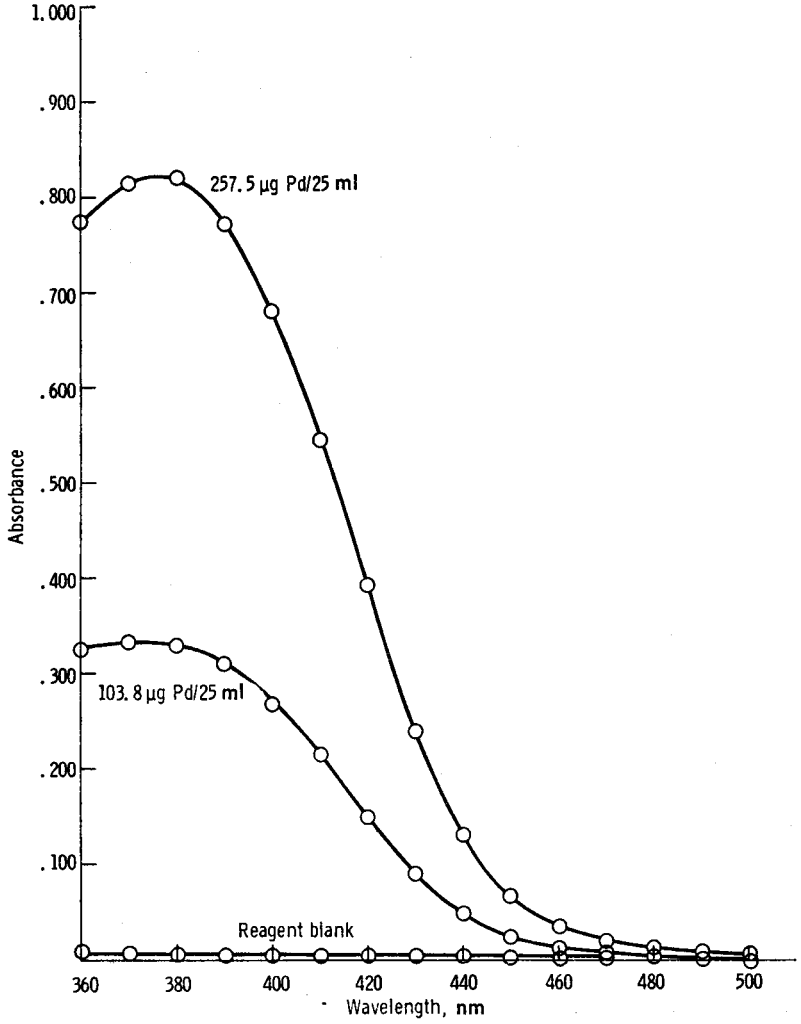

FIG. 1.—Absorption spectra of palladium dimethylglyoximate in chloroform.

Effect of pH

The palladium dimethylglyoximate complex is completely extracted in the pH range 1–6. The absorbance of the blank was found to increase, and the sensitivity for palladium to decrease, with increase in hydrochloric acid concentration below pH 1. No pH adjustment of samples is recommended as sodium hydroxide may precipitate titanium and ammonium hydroxide may affect the formation of the palladium dimethylglyoximate complex (Table I). With the procedure described, the samples extracted usually have a pH of 1–2.

TABLE I.—ANALYSIS OF Ti-BASE ALLOYS FOR PALLADIUM BY DIMETHYLGLYOXIME–EFFECT OF AMMONIA SOLUTION

Pd found, % (pH adjusted to 2·0 with NH_4OH)			Pd found, % (No pH adjustment, pH 1·5)		
C-350*	C-353†	C-357‡	C-350*	C-353†	C-357‡
0·231	0·406	0·812	0·201	0·412	0·798
0·209	0·433	0·796	0·205	0·408	0·800
0·196	0·457	0·887	0·207	0·423	0·790
0·196	0·460	0·925	0·207	0·421	0·793
			0·209	0·426	0·810
			0·208	0·428	0·794
			0·204	0·414	0·805
			0·204	0·414	0·823
		Mean	0·206	0·418	0·802
		Std. devn.	0·0027	0·0072	0·0110

* Ti-3Al-0·2Pd
† Ti-0·4Pd
‡ Ti-0·8Pd

Precision and accuracy

Precision was evaluated by repeatedly analysing solutions of several titanium alloys for palladium, using the procedure described. Two samples of a nominally 0·18% Pd-titanium alloy gave 0·190, 0·186, 0·188 and 0·190% Pd.

The results in Table I indicate a standard deviation of 27, 72 and 110 ppm for palladium contents of 2000, 4000 and 8000 ppm respectively.

Comparison of results by the dimethylglyoxime method with those obtained by the 1-nitroso-2-naphthol method of Alvarez (as described by Beamish)[14] shows fairly good agreement (Table II). It

TABLE II.—COMPARISON OF ANALYSIS OF Ti-BASE ALLOYS FOR PALLADIUM WITH DIMETHYLGLYOXIME AND 1-NITROSO-2-NAPHTHOL

Dimethylglyoxime method Pd found, %			1-Nitroso-2-naphthol method* Pd found, %		
C-349	C-354	C-358	C-349	C-354	C-358
0·202	0·410	0·828	0·193	0·398	0·809
0·199	0·410	0·828	0·196†	0·401†	0·811†
0·197	0·405	0·814	0·195†	0·401†	0·810†
0·197	0·405	0·815	0·195	0·401	0·811
		0·808*			0·809†
		0·808*			0·810
Mean 0·199	0·408	0·817	0·195	0·400	0·810

* Samples were fumed with sulphuric and perchloric acids.
† Samples were adjusted to pH 2·0 with ammonium hydroxide.

appears that fuming with sulphuric-perchloric acids may cause slightly low results. In contrast to the dimethylglyoxime method, ammonium hydroxide apparently has no adverse effect on the results obtained with 1-nitroso-2-naphthol.

Effect of diverse ions

With a potassium chloride–hydrochloric acid buffer, of the ions tested, only 5 mg of Cu(II), 10 mg of Fe(III) and 5 mg of Ni(II) gave significant deviations (Table III). Use of potassium nitrate–nitric acid buffer gave more consistent results, (Table IV), but a full statistical analysis would be needed to decide whether there is a significant difference.

TABLE III.—EFFECT OF DIVERSE IONS ON THE SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM WITH DIMETHYLGLYOXIME

Foreign ion	Pd added, μg	Pd found, μg	Deviation, μg
Ti(IV) 100 mg	28·8	28·8	0·0
Al 40 mg	28·8	28·5	−0·3
Cr(III) 10 mg	51·5	51·8	+0·3
Co(II) 10 mg	51·5	52·5	+1·0
Cu(II) 5 mg	51·5	57·3	+5·8
Cu(II) 2 mg	51·5	52·5	+1·0
Fe(III) 10 mg	51·5	53·8	+2·3
Fe(II) 50 mg	51·5	52·8	+1·3
Mo(V) 5 mg	28·8	29·2	+0·4
Ni 5 mg	51·5	58·1	+6·6
Ni 2 mg	51·5	51·5	0·0
V(V) 5 mg	51·5	52·6	+1·1
Zr 2 mg	51·5	51·5	0·0
Citric acid 1 g	25·8	25·8	0·0
Tartaric acid 1 g	25·8	25·7	−0·1
Oxalic acid 1 g	25·8	25·7	−0·1
Nitric acid, 70% 1 ml	28·8	28·8	0·0
Fluoboric acid, 48% 1 ml	28·8	28·8	0·0

TABLE IV.—COMPARISON OF USE OF CHLORIDE AND NITRATE BUFFER SOLUTIONS

Foreign ion	Recovery of 28–36 μg of Pd, %		Foreign ion	Recovery of 28–36 μg of Pd, %	
	KCl–HCl buffer*	KNO_3–HNO_3 buffer†		KCl–HCl buffer*	KNO_3–HNO_3 buffer†
Cu(II) 5 mg	101·6 103·9	100·0	V(V) 5 mg	101·6 102·7	100·0
Fe(III) 5 mg	98·9	101·1	Zr 5 mg	96·1	100·0
Ni 5 mg	101·6	98·9	Ti-6Al-4V 20 mg	97·8	98·9
Cr(III) 5 mg	102·5	101·1	Ti-4Al-4Mn 20 mg	97·8	97·8
Co(II) 5 mg	98·9	101·1	Ti-5Al-2·5Sn 20 mg	97·8	100·0
Mo(V) 5 mg	101·4	100·0	Ti-8Al-2Nb-1Ta 20 mg	98·9	98·9

* 10 ml 0·05*M* KCl–0·06*M* HCl added as buffer.
† 10 ml 0·25*M* KNO_3–0·25*M* HNO_3 added as buffer.

Tests of the recovery of palladium from chloride solutions of the noble metals gold, iridium, platinum, rhodium and ruthenium showed interference by gold and ruthenium (4 mg of metal added), but nearly normal recovery from the others. Gold caused a large positive interference while ruthenium gave negative interference because it precipitated during extraction.

Moderate amounts of any of the common mineral acids and citric, tartaric and oxalic acids have no effect on the results.

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Summary—A rapid spectrophotometric method for the determination of 0·1–1·0% of palladium in titanium alloys with dimethylglyoxime is described. The complex is extracted with chloroform and its absorbance measured at 380 nm. Beer's law is obeyed and the molar absorptivity is 170 $l.mol^{-1}.mm^{-1}$. None of the elements in common titanium alloys interfere. The method is rapid, simple and reproducible.

Zusammenfassung—Eine schnelle spektrophotometrische Methode zur Bestimmung von 0,1–1,0% Palladium in Titanlegierungen mit Dimethylglyoxim wird beschrieben. Der Komplex wird mit Chloroform extrahiert und seine Extinktion bei 380 nm gemessen. Das Beersche Gesetz gilt und der molare Extinktionskoeffizient beträgt $1{,}7 \cdot 10^3$. Keines der Elemente in den üblichen Titanlegierungen stört. Die Methode ist schnell, einfach und reproduzierbar.

Résumé—On décrit une méthode spectrophotométrique rapide pour la détermination de 0,1–1,0% de palladium dans des alliages de titane par la diméthylglyoxime. On extrait le complexe par le chloroforme et mesure son absorption à 380 nm. La loi de Beer est suivie et le coefficient d'absorption moléculaire est de $1{,}7 \times 10^3$. Aucun des éléments des alliages de titane communs n'interfère. La méthode est rapide, simple et reproductible.

REFERENCES

1. A. G. Gray, *Metal Progr.*, 1968, **94**, No. 3, 60; R. A. Wood, *DMIC Review of Recent Developments: Titanium and Titanium Alloys*, Battelle Memorial Inst., Ohio, Defense Metals and Information Center, May 1968.
2. E. B. Sandell, *Textbook of Quantitative Inorganic Analysis*, 3rd Ed., p. 85. Macmillan, New York, 1952.
3. W. Nielsch, *Z. Anal. Chem.*, 1954, **142**, 30.
4. F. E. Beamish, *The Analytical Chemistry of the Noble Metals*, p. 428. Pergamon, New York, 1966.
5. E. B. Sandell, *op. cit.*, p. 717.
6. E. W. Rice, *Anal. Chem.*, 1952, **24**, 1995; D. T. Hooker and C. V. Banks, *U.S. At. Energy Comm. Rept.* ISC-597, 1955.
7. G. H. Ayres and J. B. Martin, *Anal. Chim. Acta*, 1966, **35**, 181.
8. V. M. Peshkova, V. I. Shlenskaya, and A. I. Rashevskaya, *Vestn. Moskov. Univ.* **9**, No. 5, *Ser. Fiz. Mat. i Estestven. Nauk*, 1954, **3**, 83.
9. O. Menis and T. C. Rains, *Anal. Chem.*, 1955, **27**, 1932.
10. C. V. Banks and R. V. Smith, *Anal. Chim. Acta*, 1959, **21**, 308.
11. W. J. Holland and J. Bozic, *Anal. Chem.*, 1968, **40**, 433.
12. B. Sen, *ibid.*, 1959, **31**, 881.
13. R. T. Pflaum, M. W. Wehking, and R. E. Jensen, *Talanta*, 1964, **11**, 1193.

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ANNOTATIONS

Photometric titration of small quantities of metals with ethylenediaminetetra-acetic acid

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MICROGRAM quantities of metals are commonly determined spectrophotometrically and this procedure was preferred for the submicro determination of iron in organic materials.[1] For serial analyses, colorimetric techniques have the advantages over titrimetric procedures of having greater speed and easier manipulation. However, when single or small numbers of analyses are required, the advantage of speed largely disappears and when small numbers of different metals have to be determined the colorimetric technique suffers from the disadvantage that each metal may require its own reagents, some of which may be unstable. It was thought that an investigation of titrimetric procedures with a common titrant such as ethylenediaminetetra-acetic acid (EDTA) might provide a useful technique for work under these conditions.

Preliminary work indicated that although visual titration might be satisfactory for some low atomic weight metals, some form of instrument would be required to attain the required precision for the heavier metals. Flaschka and Sawyer[2] have discussed the use of a specially constructed phototitrator[3] for determination of small amounts of metals by complexometric titration while Headridge[4] has collated information on complexometric (mainly EDTA) titrations of metals, predominantly on the milligram scale. We have investigated the use of the commercially available EEL Titrator (Evans Electroselenium Ltd) for the photometric titration of microgram amounts of a series of metals, using freely available reagents and indicators.

General conditions used were a solution volume of 2·5–3 ml; light-path of about 14 mm; weight range of 4–20 μg of metal (except Hg, 16–32 μg); use of 0·02*M* titrant.

It was considered unreasonable to try to cover all the possible EDTA titration procedures given in the literature, owing to the vast number of indicators and variations of conditions that have been proposed. It was therefore decided to use as few different indicators and buffers as possible, consider only those titrations reported as possible at room temperature, and consider direct titrations, replacement titrations and back-titrations in that order.

EXPERIMENTAL

Selection of titration methods

Aluminium. Direct or replacement titrations at room temperature were not investigated, owing to the known slow reaction of EDTA with aluminium and the risk of forming hydroxo-complexes when neutralizing aluminium solutions. The back-titration[5] of excess of EDTA added to the aluminium solution was successful; the excess was titrated with zinc solution after buffering with hexamine, Xylenol Orange being used as indicator.

Barium. Pollard and Martin[6] used Metalphthalein for the spectrophotometric determination of barium but it was found that the difference in light absorption by the free dye and barium complex was insufficient for use with the titrator (*cf.* Cohen and Gordon[7]). Replacement and back-titrations were not considered when it was found that Methylthymol Blue[8] was satisfactory for the direct titration in dilute sodium hydroxide solution.

Bismuth. The use of Xylenol Orange,[9] at pH 2·5, was not successful; the titration curve never became linear before the end-point was reached. PAN[10] was found to be an excellent indicator for direct titration in acid solution.

Cadmium. Numerous indicators have been proposed for the direct titration of cadmium.[11] Eriochrome Black T[12] and Pyrocatechol Violet[13] did not produce sufficiently big changes in absorbance to be of use for photometric titration. Xylenol Orange[9] proved to be a satisfactory indicator, hexamine being preferred to the acetate buffer recommended elsewhere.[14]

Calcium. Methylthymol Blue[8] was tried under the same conditions as for barium and found satisfactory.

Cobalt. Unstable galvanometer readings were obtained when Murexide was used as indicator under the recommended[15] conditions. Xylenol Orange[9,16] was satisfactory.

Copper. In the presence of methanol and acetate buffer (pH 2·5),[17] precipitation occurred during the titration with PAN as indicator. This also occurred in ammoniacal solution[18] with the result that, in both conditions, the absorbance took far too long to stabilize for practical use. Insufficient colour change was found with Murexide[19] in slightly acid acetate-buffered solution, whereas in ammoniacal solution the indicator was too unstable. Xylenol Orange in acetate buffer[9,16] failed to produce a regular series of galvanometer readings after the theoretical end-point had been passed although a good straight line was given for the first part of the titration. Pyrocatechol Violet in alkaline solution[13] was not satisfactory but in hexamine-buffered solution a good titration curve was produced.

Magnesium. Eriochrome Black T[20] was first tried as a visual indicator. Reasonably satisfactory repeatability was obtained but the precision was improved by use of the photometric titrator. When the blue-green filter (603) was used, a continuous titration curve was produced. Consequently the red (608) filter, giving high absorbances at the end of the titration, had to be used.

Manganese. Pyrocatechol Violet[13] and Omega Chrome Red B[21] did not produce sufficient colour change for use with the titrator. Unstable readings were obtained for the direct titration using Eriochrome Black T[22,23] even with the addition of tartrate and ascorbic acid or hydroxylamine hydrochloride. However, a replacement titration,[24] using magnesium–EDTA and Eriochrome Black T, was successful.

Mercury. Direct titration using Xylenol Orange indicator[25] did not give the required precision, neither did the use of Methylthymol Blue.[8] Zincon has been used[26] for the absorptiometric determination of mercury but produced a suspension visible in the light beam of the titrator. A replacement titration,[27] using magnesium–EDTA and Eriochrome Black T, was reasonably satisfactory.

Nickel. Murexide has been used on the micro[24] and ultramicro[28] scales for visual titrations and was found very satisfactory for photometric titration. The Murexide–sucrose[29] solid indicator worked very well.

Zinc. Visual titrations using Eriochrome Black T or Xylenol Orange were not sufficiently repeatable on this scale but a more clearly visible end-point was observed with the latter indicator. Photometric titration with Xylenol Orange[9] (hexamine buffer) was satisfactory.

Details of the titration methods adopted are summarized in Table I. The form of the titration curve for nickel (Murexide indicator) is peculiar. No significant change in absorbance is noted until the titration has proceeded to within about 3 μl of the end-point. Recording of absorbance readings should be started as soon as one increment of EDTA decreases the absorbance by more than 0·5 scale divisions.

Reagents not specified in Table I were made up as follows:

Eriochrome Black T	0·2% in 1:1 triethanolamine/isopropanol
Methylthymol Blue	0·1% aqueous solution
Murexide	1:500 Murexide/sucrose powder
PAN	0·1% methanolic solution
Pyrocatechol Violet	0·1% aqueous solution
Xylenol Orange	0·1% in 1:1 ethanol/water
Buffer, pH 10	570 ml of conc. ammonia solution + 68 g of ammonium chloride, made up to 1 litre
Magnesium–EDTA solution	equivalent volumes (determined by titration) of 0·01*M* magnesium and EDTA solutions mixed with 5 ml of pH 10 buffer for each 200 ml of solution prepared.

Titration procedures

Apparatus. EEL Titrator (Evans Electroselenium Ltd) with 14 × 45 mm titration vessels, Ilford Spectrum filters and EEL Unigalvo Type 20. Agla syringe-burette (Burroughs Wellcome & Co).

Titrations from high to low absorbance (Type A). Adjust the galvanometer sensitivity control so that a reading of 5–10 absorbance scale division is shown with the appropriate filter and an over-titrated solution in the light-path. Add reagents and indicator to a solution containing 4–20 μg of metal in a titration vessel and make up to 2·5–3 ml with water. Place the titration vessel in the instrument and adjust the stirrer motor to give rapid stirring but without a vortex reaching the light-path. Add 0·02*M* EDTA from the syringe-burette in 0·4-μl (or 0·2-μl—see Table I) portions until the galvanometer shows an absorbance reading of about 50 divisions. From then on, record the absorbance following each addition of titrant. Continue the titration until the absorbance has remained constant, or has varied by only 0·1 division per addition, for five successive additions of titrant.

Titrations from low to high absorbance (Type B). Prepare the solution for titration as above, but adjust the galvanometer reading to zero with the untitrated solution in the light-path and the selected filter in the titrator. Add 0·02*M* EDTA in 0·4-μl portions until the absorbance reading

TABLE I.—SELECTED TITRATION METHODS

Metal	Reagents	Type of titration	Filter	Titration increments, μl
Aluminium	0·400 ml of 0·004*M* EDTA 6 drops Xylenol Orange 20 mg hexamine	Back B	605	0·2(0·02*M*/Zn)
Barium	0·2 ml 1*N* NaOH 4 drops Methylthymol Blue	Direct A	606	0·4
Bismuth	2 drops 0·5*N* HNO_3 3 drops PAN	Direct A	605	0·4 (0·2 for low amounts)
Cadmium	2 drops 0·5*N* HNO_3 3 drops Xylenol Orange hexamine to give red colour + few mg in excess	Direct A	606	0·2
Calcium	0·2 ml 1*N* NaOH 4 drops Methylthymol Blue	Direct A	606	0·4
Cobalt	1 drop 0·5*N* H_2SO_4 4 drops Xylenol Orange hexamine to give red colour + few mg in excess	Direct A	606	0·2
Copper	2 drops 0·5*N* HNO_3 2 drops Pyrocatechol Violet hexamine to give blue colour + few mg in excess	Direct A	606	0·4
Magnesium	5 drops pH 10 buffer 2 drops Eriochrome Black T	Direct B	608	0·2
Manganese	0·2 ml Mg–EDTA solution 5 drops pH 10 buffer 2 drops Eriochrome Black T	Replacement B	608	0·2
Mercury	as for manganese	Replacement B	608	0·2
Nickel	0·25 ml ammonia(1 + 1) Murexide to give good yellow colour	Direct A	601	0·4
Zinc	2 drops 0·5*N* HNO_3 6 drops Xylenol Orange hexamine to give red colour + few mg in excess	Direct A	606	0·2

changes by about 2 divisions for one addition. Readjust the galvanometer reading to zero and record the absorbance following each addition of titrant. Continue the titration until the absorbance has remained constant, or has varied by only 0·1 division per addition, for five successive additions of titrant.

Replacement titrations. Transfer 0·2 ml of magnesium–EDTA solution by pipette into the titration vessel containing the metal solution, add buffer and indicator, dilute to 2·5–3 ml and titrate as above (Type B).

Determination of end-point. Plot the absorbance against volume of titrant and draw the best straight line throught the last five points. Draw another straight line through that part of the titration curve where the absorbance differences are at a maximum and most constant, and produce this line to intersect the first one drawn. The point of intersection is taken as the end-point.

RESULTS AND DISCUSSION

Eight determinations were carried out at each of four levels of metal (nominally 4, 10, 15 and 20 μg), except for mercury, for which amounts of 16, 24 and 32 μg were taken. Bartlett's test for homogeneity of variances was applied to each of the twelve sets of data. Even though in two cases, cobalt and magnesium, heterogeneity was established by this test, the standard deviations involved in each set have been pooled to present an easily appreciated picture of the precision obtained over the whole range for each metal. These pooled standard deviations (s_g) are shown in Table II. These results are also expressed in terms of μg of metal, assuming stoichiometric reaction with the 0·02*M* titrant. Only barium and mercury exhibit standard deviations outside the 0·09–0·15 μg range although in terms of μl of titrant the results are no worse than for other metals. Their high atomic weights,

TABLE II.—RESULTS OF PHOTOMETRIC TITRATIONS

Metal	Al	Ba	Bi	Cd	Ca	Co	Cu	Mg	Mn	Hg	Ni	Zn
s_g, μl	0·28	0·07	0·04	0·06	0·11	0·11	0·10	0·18	0·10	0·11	0·11	0·08
s_g, μg	0·15	0·20	0·15	0·14	0·09	0·13	0·13	0·09	0·11	0·44	0·13	0·10
Blank, μl	1·39	1·07	0·02	0·41	1·01	0·11	0·83	0·47	0·56	0·70	0·06	0·35

however, require less scatter in the titration values (*cf.* Bi and Cd) for precision comparable with that of the lighter elements.

It is fortunate that, in the series of metals investigated, aluminium was the only metal for which a back-titration was required, so that the expected lower precision in this type of titration was not so important in terms of metal weight.

Equations of the best straight lines (calibration curves) derived from the results gave the titration blanks also shown in Table II. In the majority of cases, these blanks are too large to be explained by the uncertainty in the calibration curve. They may be due to the method of deriving the end-point of the titration or to the presence of titratable materials in the indicator and other reagents used.

The results indicate that the titrimetric finish may be suitable for the determination of metals in organo-metallic materials on the submicro scale although, owing to the presence of titration blanks, it appears to be essential that titration conditions be rigidly controlled to maintain accuracy.

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Summary—A commercially available photometric titration apparatus has been used for the determination of Al, Ba, Bi, Ca, Cd, Co, Mg, Mn, Ni and Zn in the 4–20 μg range and of Hg in the 16–32 μg range. Pooled standard deviations (32 determinations) of between 0.09 and 0.15 μg were found for all the metals except Ba (0.20 μg) and Hg (0.44 μg, 24 determinations).

Zusammenfassung—Ein käuflicher Apparat zur photometrischen Titration wurde zur Bestimmung von Al, Ba, Bi, Ca, Cd, Co, Mg, Mn, Ni und Zn im Bereich 4–20 μg und von Hg im Bereich 16–32 μg eingesetzt. Zusammengefaßte Standardabweichungen (von 32 Bestimmungen) von 0,09 bis 0,15 μg wurden gefunden bei allen Metallen außer Ba(0,20 μg) und Hg(0,44 μg, 24 Bestimmungen).

Résumé—On a utilisé un appareil de titrage photométrique commercialement disponible pour le dosage de Al, Ba, Bi, Ca, Cd, Co, Mg, Mn, Ni et Zn dans le domaine 4–20 μg et de Hg dans le domaine 16–32 μg. Les écarts types réunis (32 déterminations) ont été trouvés compris entre 0,09 et 0,15 μg pour tous les métaux à l'exception de Ba (0,20 μg) et Hg(0,44 μg, 24 déterminations).

REFERENCES

1. T. R. F. W. Fennell and J. R. Webb, *Talanta*, 1962, **9**, 795.
2. H. Flaschka and P. O. Sawyer, in *Microchemical Techniques*, *Microchem. J. Symp. Ser*, Vol. 2, p. 783. Interscience, New York, 1962.
3. *Idem*, *Talanta*, 1961, **8**, 521.
4. J. B. Headridge, *Photometric Titrations*, Chap. 5. Pergamon, Oxford, 1961.
5. M. Houda, J. Körbl, V. Bažant and R. Přibil, *Chem. Listy*, 1957, **51**, 2259; *Anal. Abstr.*, 1958, **5**, 2914.
6. F. H. Pollard and J. V. Martin, *Analyst*, 1956, **81**, 348.
7. A. I. Cohen and L. Gordon, *Anal. Chem.*, 1956, **28**, 1445.
8. J. Körbl and R. Přibil, *Chem, Listy*, 1957, **51**, 1061; *Anal. Abstr.*, 1958, **5**, 10.
9. *Idem*, *Chemist-Analyst*, 1956, **45**, 102.
10. A. I. Busev, *Zh. Analit. Khim.*, 1957, **12**, 386; *Anal. Abstr.*, 1958, **5**, 462.

11. H. Flaschka, A. J. Barnard and W. C. Broad, *Chemist-Analyst*, 1958, **47**, 22.
12. H. Flaschka and A. J. Barnard, in C. L. Wilson and D. W. Wilson, eds., *Comprehensive Analytical Chemistry*, Vol. 1B, p. 348. Elsevier, Amsterdam, 1960.
13. V. Suk and M. Malát, *Chemist-Analyst*, 1956, **45**, 30.
14. H. Flaschka and A. J. Barnard, *op. cit.*, p. 354.
15. *Idem, ibid.*, p. 351.
16. J. Körbl, R. Přibil and A. Emr, *Collection Czech. Chem. Commun.*, 1957, **22**, 961.
17. Y. Tsuchitani, Y. Tomita and K. Ueno, *Talanta*, 1962, **9**, 1023.
18. H. Flaschka and H. Abdine, *Chemist-Analyst*, 1956, **45**, 2.
19. E. M. Yakimets and N. M. Chernavina, *Tr. Ural'sk Politekhn. Inst.*, 1956, **57**, 106; *Anal. Abstr.*, 1958, **5**, 369.
20. W. Biedermann and G. Schwarzenbach, *Chemia (Switz.)*, 1948, **2**, 56.
21. R. Belcher, R. A. Close and T. S. West, *Chemist-Analyst*, 1957, **46**, 86.
22. H. Flaschka, *EDTA Titrations*, p. 77. Pergamon, London, 1959.
23. H. Flaschka and A. M. Amin, *Mikrochim. Acta*, 1953/4, 414.
24. H. Flaschka, *Mikrochemie*, 1952, **39**, 38.
25. R. Přibil and E. Körös, *Acta Pharm. Hung.*, 1957, **27**, 1; *Anal. Abstr.*, 1960, **7**, 48.
26. A. G. Morris, *Analyst*, 1957, **82**, 34.
27. N. Iritani, T. Tanaka and E. Sakai, *J. Pharm. Soc. Japan*, 1956, **76**, 1068; *Anal. Abstr.*, 1958, **5**, 400.
28. H. Flaschka and F. Sadek, *Mikrochim. Acta*, 1957, 1.
29. A. Greene, *Chemist-Analyst*, 1957, **46**, 104.

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On the atomic weight of potassium

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WITH the advent of absolute chemical measurement techniques such as coulometry, the precision of which is frequently comparable and sometimes higher (0·001%) than the uncertainty in the atomic weights, the accurate knowledge of the latter is imperative.

For many years chemical methods for the determination of combining weight ratios have been used with reasonable success for the evaluation of atomic weights. Within the last quarter of a century or so there has been a tendency to determine atomic weights through the use of mass spectrometric measurements of the isotopic abundances and values of nuclidic masses.[1] Denying the mass spectrometric methods no due credit, it should be kept in mind that reliable values of atomic weights can still be obtained by other methods, including accurate analytical methods. It is, therefore, surprising that examination of the 1961 report of the International Commission on Atomic Weights[2] shows that the atomic weight of potassium was apparently based on two sets of measurements of the abundance of ^{39}K, ^{40}K, and ^{41}K isotopes by Nier[3] and the values of the nuclidic masses from the table of Everling, Konig, Mattauch, and Wapstra.[4] (No changes in the atomic weight of potassium have been made since that time.) The atomic weight value for potassium arrived at in this manner is 39·102, which is significantly different from 39·098, the value obtained by converting the 1957 chemical atomic weight value (O = 16) 39·100 to the ^{12}C unified atomic weight scale.[5]

It appears that the combining weight ratio data obtained by Baxter and Alter,[6] Hönigschmid and Sachtleben,[7] Johnson,[8] Baxter and Harrington,[9] McAlpine and Bird,[10] were weighted very lightly in the final 1961 evaluation of the atomic weight of potassium. It is known that reliable atomic weights are obtained only if the mass spectrometer is calibrated on the "absolute" basis with specimens of accurately known isotopic ratios of the element in question, and not by making relative measurements.[1] Nier's measurements were made with the use of argon as standard.

It is the purpose of this note to draw attention to another independent source of evidence which indicates that the accepted value for the atomic weight of potassium may be somewhat high. Data obtained at the National Bureau of Standards in the differential potentiometric titrations of acids by Bates and Wichers[11] may be used for the evaluation of the atomic weight of potassium. Their comparison of single-crystal benzoic acid and potassium hydrogen phthalate with the same lot of sodium carbonate produced the following ratios:

$$\frac{C_7H_6O_2}{Na_2CO_3} = 2{\cdot}304075 \pm 0{\cdot}000051$$

$$\frac{C_8H_5O_4K}{Na_2CO_3} = 3{\cdot}853045 \pm 0{\cdot}000031$$

where the uncertainties represent standard deviations. From these values, the ratio of potassium acid phthalate to benzoic acid can readily be calculated:

$$\frac{C_8H_5O_4K}{C_7H_6O_2} = 1{\cdot}672274 \pm 0{\cdot}000050.$$

This is a new ratio, not used before for the evaluation of the atomic weight of potassium.

The atomic weight of potassium, then, may be expressed in terms of the atomic weights of C, H, and O as follows:

$$K = 3{\cdot}705918[C] + 5{\cdot}033644[H] - 0{\cdot}655452[O]$$

Using 12·01115, 1·00797, and 15·9994, for the atomic weights of C, H, and O respectively, one arrives at 39·099 (±0·001) as the value for the atomic weight of potassium.

It can be seen that this value is in much closer agreement with 39·098, the atomic weight of potassium adopted in 1957 (normalized to ^{12}C scale), than with the presently accepted value. Since the discrepancy between the presently accepted value and those obtained by other techniques cannot be reconciled with the uncertainties ascribed to each, it is hoped that more work will be stimulated to verify the accuracy of the atomic weight of potassium by other independent means.

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Summary—A brief survey of data is made, which indicates the possibility that the present internationally accepted value for the atomic weight of potassium (39·102) is too high. Additional experimental evidence is brought to light, which also supports this conclusion.

Zusammenfassung—Es wird eine kurze Übersicht über die Daten gegeben, die die Möglichkeit zeigen, daß das gegenwärtig international angenommene Atomgewicht von Kalium (39,102) zu hoch ist. Es wird noch weiteres experimentelles Material herangezogen, das diesen Schluß ebenfalls stützt.

Résumé—On procède à une brève revue de données montrant que la valeur présente internationalement acceptée pour le poids atomique du potassium (39,102) peut être trop élevée. On met en lumière des arguments expérimentaux supplémentaires qui sont également en faveur de cette conclusion.

REFERENCES

1. International Union of Pure and Applied Chemistry, *Comptes Rendus XXIV Conference*, Prague, 4–10 Sept. 1967, pp. 130–141.
2. A. E. Cameron and E. Wichers, *J. Am. Chem. Soc.*, 1962, **84**, 4175.
3. A. O. C. Nier, *Phys. Rev.*, 1950, **77**, 789.
4. F. Everling, L. A. König, S. H. E. Mattauch, and A. H. Wapstra, *Nucl. Phys.*, 1960, **18**, 529.
5. E. Wichers, *J. Am. Chem. Soc.*, 1958, **80**, 4121.
6. G. P. Baxter and Alter, *ibid.*, 1933, **55**, 3270.
7. D. Hönigschmid and R. Sachtleben, *Z. Anorg. Allgem. Chem.*, 1933, **213**, 365.
8. C. R. Johnson, *J. Phys. Chem.*, 1935, **39**, 781.
9. G. P. Baxter and C. D. Harrington, *J. Am. Chem. Soc.*, 1940, **62**, 1836.
10. R. K. McAlpine and E. J. Bird, *ibid.*, 1941, **63**, 2960.
11. R. G. Bates and E. Wichers, *J. Res. Natl. Bur. Std.*, 1957, **59**, 9.

Talanta, 1969, Vol. 16, p. i. Pergamon Press. Printed in Northern Ireland

PUBLICATIONS RECEIVED

Analysis of Organoaluminium and Organozinc Compounds: T. R. CROMPTON. Pergamon, Oxford, 1968. Pp. XIV + 354. 126s.

This book is a comprehensive review of available and potentially useful methods of analysis in organoaluminium and organozinc chemistry. Discussion of the value and applicability of each technique is followed by a careful and detailed description of experimental procedure. Consideration is given to the determination of functional groups, and to the analysis of mixtures of compounds. Discussion of a variety of titrimetric methods includes a useful chapter on thermometric titration, and the instrumental methods treated range from gas chromatography to an assessment of the analytical usefulness of infrared, Raman and N.M.R. spectroscopy. The author's claim to have brought together much hitherto unpublished material appears to be well justified in the text.

Analytical Applications of 1,10-Phenanthroline and Related Compounds: ALFRED A. SCHILT, Pergamon, Oxford, 1969. Pp. vii + 193. 70s.

This is an excellent monograph dealing with one of the more generally useful analytical reagents. It is a comprehensive review and there is a very extensive bibliography. Unlike many similar volumes the text is concise rather than discursive and the treatment is critical. The topics covered include the determination and recovery of the expensive reagents, their use as colorimetric precipitation or redox indicators and the preparation and use of a wide variety of metal chelates. The book will be invaluable to all analytical chemists.

Thermometric Titrimetry: L. S. BARK and S. M. BARK. Pergamon, Oxford, 1969. Pp. ix + 126. 45s.

This is a short monograph dealing with a relatively unfamiliar technique. The text describes experimental apparatus and the presentation of results. There are chapters dealing with neutralizations, oxidation-reduction reactions, precipitation and complex formation in aqueous systems. There is also a discussion of the application of the technique to non-aqueous solutions. The book also contains descriptions of the use of the technique in industry and an account of the information which can be derived from enthalpograms.

Reactions of Organometallic Compounds with Oxygen and Peroxides: T. G. BRILKINA and V. A. SHUSHUNOV. Iliffe, London, 1969. Pp. 225. 65s.

Analysis of Paper: B. L. BROWNING. Dekker, New York, 1969. Pp. ix + 342. $18.75.

OMR—Organic Magnetic Resonance; An International Journal. Heyden, London. Bimonthly journal; subscription £16.10 or $40.00 per annum, including Spectral Supplement.

Sodium Hydride: Its Use in the Laboratory and in Technology: J. PLESEK and S. HERMANEK. Iliffe, London, 1969. Pp. 185. 45s.

Radiochemical and Radioanalytical Letters. Elsevier Sequoia, Lausanne. Two volumes per year. Subscription £21. 10 or $50.00 per annum.

Reprints of the following reviews published in Talanta are available from Learned Journals Division, Pergamon Press Ltd., Headington Hill Hall, Oxford, England, or Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.

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31 **Applications of Digital Computers in Analytical Chemistry—I.** by C. W. Childs, P. S. Hallman and D. D. Perrin

32 **Applications of Digital Computers in Analytical Chemistry—II.** by C. W. Childs, P. S. Hallman and D. D. Perrin

33 **Fluorescence Analysis in Air Pollution Research** by E. Sawicki

SUMMARIES FOR CARD INDEXES

Fluorescence analysis in air pollution research: E. SAWICKI, *Talanta*, 1969, **16**, 1231. (U.S. Department of Health, Education and Welfare, Consumer Protection and Environmental Health Service, National Air Pollution Control Administration, Chemical and Physical Research and Development Program, 4676 Columbia Parkway, Cincinnati, Ohio 45226, U.S.A.)

Summary—Luminescence phenomena are of value in the analysis of air pollutants. The problems arising in the use of excitation and emission spectra under various conditions are discussed. Phenomena such as solvent, pH, and photochemical effects are shown to play an important role in the fluorimetric analysis of air pollutants. Many of the fluorimetric methods used in the trace analysis of organic airborne particulates involve factors such as direct measurement of the separated pollutant on a chromatogram or pherogram, quenching phenomena, scanning, excimer formation, charge-transfer fluorescence, sensitized fluorescence, and photo-oxidation on adsorbent or in solution. In addition, fluorescence assay methods are discussed in terms of selectivity, sensitivity, speed, simplicity, accuracy, precision, interferences, and the relation between concentration and fluorescence intensity.

Catalytic titrants and catalytic indication of end-points: HORACIO A. MOTTOLA, *Talanta*, 1969, **16**, 1267. (Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma 74074, U.S.A.)

Summary—Methods of analysis based on rate measurement or exploiting properties of systems not at equilibrium are becoming important in analytical chemistry. Kinetic and equilibrium considerations of catalytic titrants and catalytic end-point detection are presented and discussed. Applications of the approach to the determination of major and trace components of organic and inorganic species are included and discussed.

ПРИМЕНЕНИЕ ФЛУОРЕСЦЕНТНОГО АНАЛИЗА В ИССЛЕДОВАНИИ ЗАГРЯЗНЕНИЯ ВОЗДУХА:

E. Sawicki, *Talanta*, 1969, **16**, 1231.

Резюме—Люминесценция является очень важным феноменом в анализе загрязняющих воздух веществ. Обсуждены проблемы связанные с применением спектров возбуждении и эмиссии в разных условиях. Факторы как на пример растворитель, pH и фотохимические эффекты влияют в значительной мере на определение загрязняющих воздух веществ методом флуориметрического анализа. В ряде методов флуориметрического анализа использованных для следового анализа органических частиц в воздухе включено прямое определение выделенного загрязнителя на хроматограмме или ферограмме, погашение флуоресценции, развитие хроматограмм или спектров, образование эксцимеров, флуоресценция вызванная переносом заряда, сенсибилизированная флуоресценция и фотоокисление на адсорбенте или в растворе. Обсуждены селективность, чувствительность, быстрота, несложность, точность, воспроизводимость методов флуоресцентного анализа, мешающие влияния и отношение между концентрацией и интенсивности флуоресценции.

КАТАЛИТИЧЕСКИЕ ТИТРОВАННЫЕ РАСТВОРЫ И КАТАЛИТИЧЕСКОЕ ИНДИЦИРОВАНИЕ КОНЦА ТИТРОВАНИЯ:

Horacio A. Mottola, *Talanta*, 1969, **16**, 1267.

Резюме—В настоящее время придается значение аналитическими методами, которые основаны на измерении скорости реакции или которые пользуются характеристиками систем не находящихся в равновесии. Рассмотрены с точки зрения кинетики и равновесия каталитические титрованные растворы и каталитическое обнаружение конца титрования. Приведены и обсуждены примеры применения метода в определении больших и следовых количеств органиеских и неорганических веществ.

Acidity of several chromotropic acid azo derivatives: B. BUDĚŠÍNSKÝ, *Talanta*, 1969, **16**, 1277. (Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada.)

Summary—Both potentiometric and spectrophotometric methods have been used for the determination of the stability constants of hydrogen complexes of 4,5-dihydroxynaphthalene-2,7-disulphonic acid (Chromotropic Acid, CA), 3,6-bis(phenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Azo III, A III), 3,6-bis(2′-sulphophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Sulphonazo III, SA III), 3,6-bis(4′-methyl-2′-sulphophenylazo)-4,5-dihydroxynaphthalene 2,7-disulphonic acid (Dimethylsulphonazo III, DMSA III), 3-(4′-chloro-2′-phosphonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Chlorophosphonazo I, CPA I), 3,6-bis(4′-chloro-2′-phosphonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Chlorophosphonazo III, CPA III), 3-(2′-arsonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Arsenazo I, AA I) and 3,6-bis(2′-arsonophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Arsenazo III, AA III).

Differential reflectance spectrophotometry—I. High-reflectance method for determination of micro amounts of substances resolved on thin plates: VAN T. LIEU, DAVID F. ZAYE and MICHAEL M. FRODYMA, *Talanta*, 1969, **16**, 1289. (Department of Chemistry, University of Hawaii, Honolulu, Hawaii, U.S.A.)

Summary—The basic principles of differential high-reflectance spectroscopy are discussed from the standpoint of the determination of substances resolved on chromatoplates. Results obtained with the use of two systems, nickel dimethylglyoximate or copper neocuproinate adsorbed on cellulose, are used as illustrations. A graphical method for selecting the optimum concentration range for analysis and for determining the maximum accuracy attainable is also outlined. When contrasted with the conventional method of measuring reflectance, the technique promises substantially increased accuracy over a wider concentration range and seems particularly suited to the analysis of trace amounts of material.

КИСЛОТНОСТЬ НЕКОТОРЫХ АЗОПРОИЗВОДНЫХ ХРОМОТРОПОВОЙ КИСЛОТЫ:

B. Buděšínský, *Talanta*, 1969, **16**, 1277.

Резюме—Методы потенциометрического и спектрофотометрического анализа использованы для определения констант устойчивости водородных комплексов 4,5-диоксинафталин-2,7-дисульфоновой кислоты (хромотроповой кислоты, ХК), 3,6-бис(фенилазо)-4,5-диоксинафталин-2,7-дисульфоновой кислоты (Азо III, А III), 3,6-бис(2′-сульфофенилазо)-4,5-, диоксинафталин-2,7-дисульфоновой кислоты (Сульфоназо III-СА III), 3,6-бис(4′-метил-2′-сульфофенилазо)-4,5-диоксинафталин-2,7-дисульфоновой кислоты (Диметилсульфоназо III, ДМСА III), 3-(4′-хлор-2′-фосфонофенилазо)-4,5-диоксинафталин-2,7-дисульфоновой кислоты (Хлорфосфоназо I, ХФА I), 3,6-бис(4,-хлор-2,-фосфонофенилазо)-4,5-диоксинафталин-2,7-дисульйоновой кислоты (Хлорфосфоназо III, ХФА III), 3-(2′-арсонофенилазо)-4,5-диоксинафталин-2,7-дисульфоновой кислоты (Арсеназо I, АА I) и 3,6-бис(2′-арсонофенилазо)-4,5-диоксинафталин-2,7-дисульфоновой кислоты (Арсеназо III, АА III).

ДИФФЕРЕНЦИАЛЬНАЯ ОТРАЖАТЕЛЬНАЯ СПЕКТРОФОТОМЕТРИЯ—I. МЕТОД ВЫСОКОГО ОТРАЖЕНИЯ ДЛЯ ОПРЕДЕЛЕНИЯ МИКРОГРАММОВЫХ КОЛИЧЕСТВ ВЕЩЕСТВ РАЗДЕЛЕННЫХ НА ПЛАСТИНКАХ ТОНКОСЛОЙНОЙ ХРОМАТОГРАФИИ:

Van T. Lieu, David F. Zaye and Michael M. Frodyma, *Talanta*, 1969, **16**, 1289.

Резюме—Обсуждены основные принципы дифференциальной высокоотражательной спектроскопии с целью применения метода в определении веществ разделенных на тонкослойных хроматограммах. Метод иллюстрирован результатами полученными в двух системах—диметилглиоксимате никеля или неокупроинате меди адсорбированных на целлюлозе. Также приведен графический метод отбора оптимальных пределах концентрации для анализа и определения максимальной иолучаемой точности. В сравнении с обыкновенным методом шзмерения отражения, новый метод дает значительно повышение точности в широкой области концентраций и является особенно подходящим для анализа следовых количеств материала.

Activation analysis for mercury in biological samples at nanogram level: L. KOSTA and A. R. BYRNE, *Talanta*, 1969, **16**, 1297. (Faculty of Natural Sciences, University of Ljubljana and Nuclear Institute "Jožef Stefan", Ljubljana, Yugoslavia.)

Summary—A new method has been devised for determining mercury in samples of biological origin. It is based on complete ignition of the sample in a silica tube, trapping volatile interfering activities such as bromine or chlorine, and selectively adsorbing mercury on a strip of filter paper which has been previously impregnated with elemental selenium. This strip is later counted for quantitative evaluation. The versatility of the method has been demonstrated by the analysis of a wide range of samples such as water, cellulose, flour, fish solubles or animal blood samples with mercury contents between 1 and 200 ng/g of sample.

Determination of cobalt and zinc in high-purity niobium, tantalum, molybdenum and tungsten metals by atomic-absorption spectrophotometry after separation by extraction: ELSIE M. DONALDSON, D. J. CHARETTE and VERA H. E. ROLKO, *Talanta*, 1969, **16**, 1305. (Mineral Sciences Division, Department of Energy, Mines and Resources, Mines Branch, Ottawa 4, Ontario, Canada.)

Summary—A method for determining 0·0005–0·05% of cobalt and zinc in high-purity niobium, tantalum, molybdenum and tungsten metals by atomic-absorption spectrophotometry is described. After sample dissolution, cobalt and zinc are separated simultaneously from the matrix materials by chloroform extraction of their thiocyanate–diantipyrylmethane ion-association complexes, at pH 3·25, from a citric acid medium approximately 1·2*M* in sodium thiocyanate. Interference from copper is eliminated with thiourea. Large amounts of iron interfere under the recommended conditions, but moderate amounts may be present in the sample solution without causing appreciable error in the results. Phosphorus (as orthophosphate) interferes in the extraction of cobalt from tungsten solutions. Moderate amounts of other impurities do not interfere in the proposed method.

ОПРЕДЕЛЕНИЕ НАНОГРАММОВЫХ КОЛИЧЕСТВ РТУТИ В БИОЛОГИЧЕСКИХ ВЕЩЕСТВАХ МЕТОДОМ РАДИОАКТИВАЦИОННОГО АНАЛИЗА:

L. Kosta and A. R. Byrne, *Talanta*, 1969, **16**, 1297.

Резюме—Разработан новый метод определения ртути в образцах биологического происхождения. Метод основан на обзоивании образца в кварцевой трубке, задержая летучие мешающие активности, как на пример бром или хлор, и на селективной адсорбции ртути на ленте фильтровальной бумаги предварительно пропытанной элементарным селеном. Эту ленту считают для количественного определения. Многосторонность метода показана его применением в анализе разных образцов, как на пример воды, целлюлозы, муки, растворимой части рыб и крови животных, содержащих 1–200 нг/г ртути.

ОПРЕДЕЛЕНИЕ КОБАЛЬТА И ЦИНКА В ВЫСОКОЧИСТОТНЫХ МЕТАЛЛАХ НИОБИИ, ТАНТАЛЕ, МОЛИБДЕНЕ И ВОЛЬФРАМЕ МЕТОДОМ АТОМНОАБСОРБЦИОННОЙ СПЕКТРОФОТОМЕТРИИ ПОСЛЕ ВЫДЕЛЕНИЯ ЭКСТРАКЦИОННЫМ МЕТОДОМ:

Elsie M. Donaldson, D. J. Charette and Vera H. E. Rolko, *Talanta*, 1969, **16**, 1305.

Резюме—Описан метод определения 0,005–0,05% кобальта и цинка в высокочистотных металлах ниобии, тантале, молибдене и вольфраме методом атомно-абсорбционной спектрофотометрии. Пробу растворяют, а кобальт и цинк выделяют одновременно из матричного материала извлеченимых роданиддиантипирилметановых ионноассоциационных комплексов с хлороформом при pH 3,25, из лимоннокислой среды содержащей 1,2*M* роданида натрия. Влияние меди исключено добавкой тиомочевины. В предложенных условиях большие количества железа мешают определению, но умеренные количества можно толерировать в растворе пробы—они не влияют на результаты в значительной мере. Фосфор (в форме ортофосфата) мешает извлечению кобальта из растворов вольфрама. Умеренные количества других примесей не мешают предложенному методу.

Coulometric titration of bases in acetic acid and acetonitrile media VILIM J. VAJGAND and RANDJEL MIHAJLOVIĆ, *Talanta*, 1969, **16**, 1311. (Institute of Chemistry, Faculty of Sciences, University of Belgrade, Studentskii trg 16, Yugoslavia.)

Summary—The working conditions and the results for coulometric titration of milligram amounts of some bases in 0.1*M* sodium perchlorate in a mixture of acetic acid and acetic anhydride (1:6), are given. Determinations were made both by coulometric back-titration or direct titration at the platinum anode. Back-titration was done in the catholyte, by coulometric titration of the excess of added perchloric acid. The titration end-point was detected photometrically with Crystal Violet as indicator. The direct titration of bases was done at the platinum anode, in the same electrolyte, to which hydroquinone was added as anode depolarizer and as the source of hydrogen ions, Malachite Green being used as indicator. Similarly, bases can be determined in acetonitrile if sodium perchlorate, hydroquinone and Malachite Green are added to the solvent. Errors are below 1%, and the precision is satisfactory.

Potentiometric determination of successive stability constants of ethylenediamine complexes of several metals in dimethylsulphoxide: KARL H. POOL and DONALD E. SANDBERG, *Talanta*, 1969, **16**, 1319. (Department of Chemistry, Washington State University, Pullman, Washington 99163, U.S.A.)

Summary—The stability constants of the complexes of silver(I), zinc(II), cadmium(II) and manganese(II) with ethylenediamine in dimethylsulphoxide at 25° and ionic strength 0·1*M* have been determined potentiometrically. The stability constants are consistently larger in DMSO than in water as expected from the difference in dielectric constant for the two solvents.

КУЛОНОМЕТРИЧЕСКОЕ ТИТРОВАНИЕ ОСНОВАНИЙ В УКСУСНОЙ КИСЛОТЕ И АЦЕТОНИТРИЛЕ:

VILIM J. VAJGAND and RANDJEL MIHAJLOVIĆ, *Talanta*, 1969, **16**, 1311.

Резюме—Приведены опытные условия и результаты кулонометрического титрования миллиграммовых количеств некоторых оснований в 0,1*M* перхлорате натрия в смеси уксусной кислоты и ангидрида уксусной кислоты (1:6). Определения проведены кулонометрическим оттитровыванием избытка или прямым титрованием на платиновом электроде (аноде). Оттитровывание избытка проведено в католите, кулонометрическим оттитровыванием избытка добавленной хлорной кислоты. Конец титрования обнаружен фотометрическим методом с использованием кристаллфиолетового в качестве индикатора. Прямое титрование оснований проведено на платиновом аноде в том же электролите, которому добавили гидрохинон в качестве анодного деполяризатора и источника ионов водорода; малахитовым зелёным пользовались в качестве индикатора. Схожим путем основания можно определять в ацетонитриле, добавлением гидрохинона и малахитового зелёного к растворителе. Ошибки меньше чем 1%, а воспроизводимость удовлетворительная.

ПОТЕНЦИОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ПОСЛЕДУЮЩИХ КОНСТАНТ УСТОЙЧИВОСТИ КОМПЛЕКСОВ ЭТИЛЕНДИАМИНА С НЕКОТОРЫМИ МЕТАЛЛАМИ В ДИМЕТИЛСУЛЬФОКСИДЕ:

KARL H. POOL and DONALD E. SANDBERG, *Talanta*, 1969, **16**, 1319.

Резюме—Определены потенциометрическим методом константы устойчивости комплексов серебра(I), цинка(II), кадмия(II) и марганца(II) с этилендиамином в диметилсульфоксиде при 25° и ионной силы 0,1*M*. Все константы устойчивости больше в ДМСО чем в воде, как ожидалось на основе разници между диэлектрческими константами этих двух растворителей.

Determination of cerium in minerals with sulphanilic acids: E. N. POLLOCK, *Talanta*, 1969, **16**, 1323. (Ledgemont Laboratory, Kennecott Copper Corporation, Lexington, Massachusetts 02173, U.S.A.)

Summary—Cerium can be determined colorimetrically in minerals with sulphanilic acid. Cerium(III) ions are oxidized with silver(II) in 1–7% sulphuric acid. Sulphanilic acid is oxidized by cerium(IV) ions in 20% sulphuric acid. The absorbance is determined at 540 nm. In the presence of maganese or chromium, cerium can be separated by precipitation as the oxalate. Lanthanum can be used as a gathering agent if necessary.

Determination of uranium in rocks by instrumental activation-analysis using epithermal neutrons: E. STEINNES and D. BRUNE, *Talanta*, 1969, **16**, 1326. (Institutt for Atomenergi, Isotope Laboratories, Kjeller, Norway.)

Summary—A rapid non-destructive neutron-activation method for the determination of uranium in rocks is described. The method is based on activation with epithermal neutrons and subsequent measurement of the 74 keV γ-ray of ^{239}U. Results given for some standard rocks are in good agreement with literature data. The precision of the method is about 5% and the limit of detection is of the order of 0.1 ppm in silicate rocks.

Spectrophotometric determination of palladium in titanium-base alloys, with dimethylglyoxime: WARREN F. DAVIS, *Talanta*, 1969, **16**, 1330. (Lewis Research Center, National Aeronautics and Space Administration, Cleveland, Ohio, U.S.A.)

Summary—A rapid spectrophotometric method for the determination of 0·1–1·0% of palladium in titanium alloys with dimethylglyoxime is described. The complex is extracted with chloroform and its absorbance measured at 380 nm. Beer's law is obeyed and the molar absorptivity is 170 $l.mol^{-1}.mm^{-1}$. None of the elements in common titanium alloys interfere. The method is rapid, simple and reproducible.

ОПРЕДЕЛЕНИЕ ЦЕРИЯ В МИНЕРАЛАХ СУЛЬФАНИЛОВОЙ КИСЛОТОЙ:

E. N. Pollock, *Talanta*, 1969, **16**, 1323.

Резюме—Церий определен в минералах колориметрическим методом с использованием сульфаниловой кислоты. Ионы церия(III) окисляют серебром(II) в 1–7% серной кислоте. Сульфаниловую кислоту окисляют церием(IV) в 20% серной кислоте. Светопоглощение измеряют при 540 нм. Церий отделяют от марганца или хрома осаждением в форме оксалата. Если нужно лантаном можно пользоваться в качестве накопляющего агента.

ОПРЕДЕЛЕНИЕ УРАНА В ГОРНЫХ ПОРОДАХ ИНСТРУМЕНТАЛЬНЫМ МЕТОДОМ АКТИВАЦИОННОГО АНАЛИЗА С ИСПОЛЬЗОВАНИЕМ ЭПИТЕРМАЛЬНЫХ НЕЙТРОНОВ:

E. Steinnes and D. Brune, *Talanta*, 1969, **16**, 1326.

Резюме—Описан быстрый недеструктивный метод нейтронно-активационного анализа для определения урана в горных породах. Метод основан на активации эпитермальными нейтронами и последующим измерением 74 кэв гамма-лучей ^{239}U. Результаты полученные для некоторых стандартных породах хорошо соглашаются с литературными данными. Точность метода 5%, а чувствительность порядка 0,1 мкг/г в силикатных горных породах.

СПЕКТРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ПАЛЛАЦИЯ В СПЛАВАХ НА ОСНОВЕ ТИТАНА, С ДИМЕТИЛГЛИОКСИМОМ:

Warren F. Davis, *Talanta*, 1969, **16**, 1330.

Резюме—Описан быстрый спектрофотометрический метод определения 0,1–1,0% палладия в сплавах титана с диметилглиоксимом. Комплекс извлекают хлороформом и измеряют его светопоглощение при 380 нм. Закон Бера почитается, а молярное поглощение равно $1{,}7 \times 10^3$. Обыкновенные элементы в сплавах титана не мешают определению. Метод является быстрым, несложным и воспроизводимым.

Photometric titration of small quantities of metals with ethylenediaminetetra-acetic acid: R. BELCHER, BARBARA CROSSLAND and T. R. F. W. FENNELL, *Talanta*, 1969, **16**, 1335. (Department of Chemistry, Birmingham University, P.O. Box 363, Birmingham 15, U.K.)

Summary—A commercially available photometric titration apparatus has been used for the determination of Al, Ba, Bi, Ca, Cd, Co, Mg, Mn Ni and Zn in the 4–20 μg range and of Hg in the 16–32 μg range. Pooled standard deviations (32 determinations) of between 0.09 and 0.15 μg were found for all the metals except Ba (0.20 μg) and Hg (0.44 μg, 24 determinations).

On the atomic weight of potassium: GEORGE MARINENKO, *Talanta*, 1969, **16**, 1339. (Division of Analytical Chemistry, Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234, U.S.A.)

Summary—A brief survey of data is made, which indicates the possibility that the present internationally accepted value for the atomic weight of potassium (39·102) is too high. Additional experimental evidence is brought to light, which also supports this conclusion.

ФОТОМЕТРИЧЕСКОЕ ТИТРОВАНИЕ НЕБОЛЬШИХ КОЛИЧЕСТВ МЕТАЛЛОВ ЭТИЛЕНДИАМИНТЕТРАУКСУСНОЙ КИСЛОТОЙ:

R. Belcher, Barbara Crossland and T. R. F. W. Fenell, *Talanta*, 1969, 16, 1335.

Резюме—Имеющийся в продаже прибор для фотометрического титрования использован для определения Al, Ba, Bi, Ca, Cd, Co, Mg, Mn, Ni и Zn в пределах 4–20 мкг, а Hg в пределах 16–32 мкг. Получены объединенные стандартные ошибки (32 определения) от 0,09 до 0,15 мкг для всех металлов кроме Ba (0,20 мкг) и Hg (0,44 мкг, 24 определений).

О АТОМНОМ ВЕСЕ КАЛИЯ:

George Marinenko, *Talanta*, 1969, **16**, 1339.

Резюме—Приведен короткий обзор данных, указывающих на возможность что международно принятая величина атомного веса калия (39,102) слишком высокая. Приведены так же экспериментальные данные подкрепляющие этот вывод.

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