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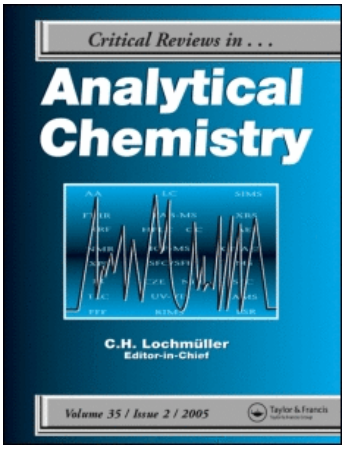

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

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To cite this Article Łobiński, Ryszard and Marczenko, Zygmunt(1992) 'Recent Advances in Ultraviolet-Visible Spectrophotometry', Critical Reviews in Analytical Chemistry, 23: 1, 55 — 111
To link to this Article: DOI: 10.1080/10408349208051647
URL: http://dx.doi.org/10.1080/10408349208051647

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Critical Reviews in Analytical Chemistry, 23(1):55–111 (1992)

Recent Advances in Ultraviolet-Visible Spectrophotometry

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ABSTRACT: Developments in UV/VIS spectrophotometry during the past decade are critically reviewed. Advances in instrumentation: sample handling, optical systems, detectors, and signal processing are discussed. The application of spectrophotometric detection in hydrodynamic systems is surveyed. Newer types of color systems employing macrocyclic compounds, porphyrines, surfactants, and basic dyes are evaluated. Separate sections are devoted to advances in spectrophotometric determination both of individual elements and organic compounds. Future trends in spectrophotometric analysis are discussed.

KEY WORDS: spectrophotometry, FIA, liquid chromatography, kinetic methods, solid-phase spectrophotometry, multiwavelength analysis, derivative spectrophotometry, macrocyclic compounds, pyridylazo compounds, porphyrines, surfactants, flotation-spectrophotometry, element determination.

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I. INTRODUCTION

Ten years ago, an article devoted to spectrophotometric determination of trace elements was published in this journal by Marczenko.[1] Since then, atomic absorption and plasma atomic emission analysis have rapidly developed and during the past decade have diminished the role of spectrophotometry as the most widely used analytical technique in many laboratories. Indeed, generally lower detection limits and enhanced selectivity of atomic spectrometric techniques, together with the ease of sequential multielement determination capabilities, make these techniques better suited for metals determination than spectrophotometry. Spectrophotometry, however, continues to enjoy wide popularity. The common availability of the instrumentation and the simplicity of procedures as well as speed, precision, and accuracy of the technique still make photometric methods an attractive alternative, resulting in extensive literature being published. A number of books and general review articles dealing with various aspects of theory and methodology and also containing a useful compilation of practical information relative to the determination of elements have appeared.[1–17]

Considerable advances have been made in spectrophotometric techniques during the past 10 years, which have created new application areas. The advent of a new generation of spectrophotometers equipped with diode array detectors and extensive use of microprocessors in data aquisition and handling have brought about dynamic progress in the simultaneous analysis based on multiwavelength and derivative measurements. This helped the trend from inorganic toward organic spectrophotometric analysis, which is reflected in three published books.[18–20] Spectrophotometry has become the most widely used detection technique in flow-injection analysis (FIA) and its use in chromatography has gained considerable momentum. The last, but certainly not least, area of progress to be mentioned is the development of new more sensitive color systems based either on new reagents (crown ethers, por-

Note: Manuscript solicited by Walter L. Zielinski, Jr.

1040-8347/92/$.50
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phyrines) or on powerful combinations of existing ones (mixed ligand ternary and quaternary complexes, micellar systems, and flotable ion-associates). Organic analytical reagents were the subject of one book[21] and a comprehensive dictionary is being prepared.[22]

The aim of this article is to highlight recent advances and trends in spectrophotometry in a critical manner. Recent developments both in analytical instrumentation and techniques as well as in the chemistry of sensitive color systems for spectrophotometry are discussed. Separate sections are devoted to a survey of new methods for the determination both of individual elements and organic compounds, including relevant separation techniques and areas of application.

II. ADVANCES IN SPECTROPHOTOMETRIC TECHNIQUES

A. Apparatus

Spectrophotometric instrumentation has undergone continuous development. Virtually no part of the spectrophotometer has remained unchanged since the late 1970s. The history of the evolution of the apparatus for ultraviolet-visible (UV/VIS) spectrophotometry from the early days through the mid-1980s was described.[23,24] Here, a brief account of the current state of the art is given, with particular attention being paid to those developments that either have already become a standard in analytical laboratories or, in our opinion, are expected to do so in near future.

1. Sample Handling

A significant trend in sample handling is increased automation. Modern spectrophotometers are equipped with sample compartments that accept cuvettes of various pathlengths and size. Microprocessor-controlled cell holders load and measure a number of samples sequentially, thus saving the operator's time. More spectrophotometers are interfaced with an autosampling device capable of handling (via the flow-through cell) a large number of samples (up to a few hundred) contained in test tubes. The autosampler is often used in combination with a set-up for automatic dispensing and mixing reagents, diluting samples, and filling the absorption cell. Other "intelligent" devices for performing the preparation of the sample from the very beginning, i.e., involving weighing, mixing, dissolving, and even carrying out chemical reactions and separations, are soon expected. At present, many microprocessor-controlled systems are available that can run the analysis continuously and unattended thus resulting in improved precision due to the elimination of operator error. The trend has developed into a completely new area of sample processing — FIA — discussed later in a separate section.

2. Light Sources

Organic constituents are preferably determined with UV light sources, while in inorganic spectrophotometric analysis, the visible part of the absorption spectrum is usually utilized.

Advances in UV sources were recently discussed.[25] The deuterium lamp is mainly used for this wavelength range due to its high-energy continuous light emission, good temporal and long-term stability, and lifetime. For the visible range, tungsten-halogen lamps have replaced tungsten ones due to their longer life and larger energy output. For dual lamp spectrophotometers, the further optimization of the lamp interchange mirror and light source geometry ensured higher energy and lower noise throughout the UV/VIS range. Although most spectrophotometers still use two independent light sources for UV and VIS ranges, recent instrumentation uses only a single one, especially in systems with reverse optics. New developments in deuterium lamp technology made it possible to obtain emission in the 190- to 820-nm range thereby covering the UV/VIS range spectrophotometry requirements.[26] The use of broad band mixed-alkali-metal photocathodes (160 to 900 nm)[27] and mercury-xenon sources[28] for the same purpose was reported.

An interesting development shows discrete radiation sources such as light-emitting diodes (LEDs). They offer an exceptionally simple and inexpensive source of monochromatic light, which can be employed in field portable photometric

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monitors as well as in other automated systems. The applications of LEDs are still limited due to the small number of available wavelengths of the emitted light. Applications of this method are, however, expected to grow because the large number of organic reagents available makes it relatively easy to find a suitable color system absorbing the appropriate wavelength. The principle of operation of LEDs was described and the applications were reviewed.[29]

The most recent trend in light source development is represented by the increasing use of lasers. Their development and wider availability gave birth to a new group of sensitive techniques such as photoacoustic and thermal lens spectrophotometry, which are discussed in more detail later. Also, in conventional spectrophotometry, the advantages of lasers, such as the high-emission intensity and the monochromaticity of light, are likely to bring advances in sensitivity and spectral resolution soon. Lasers are one of the best sources for detectors in chromatography because the laser beam can easily be focused on the tiny spot inside the capillary orifice.[30] Tunable diode lasers, which were recently reviewed,[31] are mostly used. They have many advantages over conventional lasers such as smaller size and longer life, but their emission wavelength is restricted to 670 nm. For this reason, their application in spectrophotometry to date is confined to a few areas, the most common being monitoring atmospheric pollution.[32,33] A wider availability of shorter wavelength diode lasers will probably bring rapid progress in the near future.

3. Optical Systems

Until the late 1970s, scanning across the spectral region of interest was the only way to obtain spectrometric information on the sample. The development of detectors capable of simultaneously monitoring all wavelengths of the spectrum brought about dramatic changes in the optical systems of commercially available spectrophotometers. Their new generation applying reverse optics (i.e., with the diffractor placed between the sample cell and the detector and not between the light source and sample cell) came into being.

At the same time, important developments were made in spectrophotometers utilizing forward optics. As far as optical geometry is concerned, single-beam instruments have become obsolete. They show considerable disadvantages such as poor stability and excessive drift because the reference and the sample scans are separated in time. Dual-beam optics designs eliminating these drawbacks have grown into the standard in today's UV/VIS spectrophotometers. The primary light beam is split after the grating into two separate beams, one of which passes through the sample and the other passes through the reference. The same lamp image is thus created at both the reference and the sample photodiodes, thus eliminating time-dependent irregularities in the source, detector, and associated electronics. The result is extremely low noise and drift, which are not possible even with reverse optics design. Double-beam instruments, however, have considerable disadvantages originating in the complex construction, high cost, and low light efficiency because only half of the emitted energy is utilized at the sample. An innovative design of split beam optics was proposed as a cost-effective trade-off between single- and double-beam optics.[34] Only a small fraction of the emitted light is used as the reference beam while the majority is directed through the sample. This design allows for better utilization of the emitted light while maintaining constant resolution. It also offers fast response, better stray light elimination, and a high signal-to-noise ratio.

As far as the diffractor is concerned, gratings have proved to be superior over prisms in providing stable, parallel, and highly monochromatic light due in part to constant wavelength dispersion. Moreover, they are of a relatively small size and offer a large working range from UV to near IR. New developments in technology have enabled the production of gratings by holographic techniques. Due to freedom from mechanical inaccuracies, they are capable of virtually eliminating stray radiant energy. Good efficiency of the monochromator together with the high-energy output of the light source and the development of sensitive photodetectors

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helped to reduce the bandwidth of the currently available spectrophotometers down to a few nanometers.

To scan a sample over a range of wavelengths, the grating must be moved to obtain all the wavelengths desired. The manual adjustment of wavelengths was replaced long ago by a drive allowing for automatic measurements over a specified wavelength range. With classic photometers, the scanning is mechanical and the reproducibility of the grating position is poor. Furthermore, the dependence of wavelength on the movement of the grating is not linear and cannot be well compensated for with a mechanical setup. The latest developments allow for rapid, precise positioning of the holographic grating using laser beams. This technology, called low-inertia scanning, is based on precise electromagnetic movement of the grating and offers extreme accuracy and unfailing reliability (30-nm/s scanning speed with <0.001-nm precision). The operation of the system is controlled by microprocessors, which select the lamp and the lamp interchange mirror (in two lamp instruments), position the filter wheel and the grating, and control the wavelength autocalibration by the instrument.

Both forward and reverse optics designs have their advantages and their limitations. Forward optics provide optical performance superior to diode array reverse optics due to better elimination of stray light effects and ease of compensation for source and detector drift in dual-beam instruments. On the other hand, the simplicity of the optical system in reverse optics designs (only three optical parts) results in improving the detection limits of the instrument due to a larger fraction of the source light reaching the detector. Furthermore, scanning photometers are not ideally suited for on-line analysis because their scanning time is slow relative to the flowing process stream. Moreover, they can exhibit reproducibility and reliability problems associated with the moving parts. For these reasons, the reverse optics instruments enjoy wider popularity at the moment. However, the introduction of the low-inertia scanning technology on a larger scale will not only make rapid scanning possible with forward optics instruments, but also will bring about appreciably higher precision than is exhibited so far by photodiode array instruments.

4. Cuvettes

In conventional spectrophotometry, quartz cuvettes for the UV and plastic disposable ones for the VIS range are now standard. They are available in sets of variable pathlength, usually from 5 μm to 10 cm. Microcells are used when only a small sample volume (up to 100 μl) is available.

Recent studies have focused on the development of cells containing a small sample volume but providing extremely long pathlengths, such as hollow glass and quartz fibers. Pathlengths of up to 130 m could be obtained.[35,36] Another possibility are multipath cells. Instead of exploiting very long pathlengths, they take advantage of the effect of multiple pathlengths. Unlike the multipass cell commonly used with gases, which involves multiple reflection of a single beam traversing the cell, in a liquid multipath cell, the effective pathlength varies with solution absorbance. The pathlength is substantially greater for solutions of low rather than of high absorbance, thus extending the dynamic range of absorbance measurements. The multipath cells described include a 10-cm cuvette, which uses a variable path (10 mm to 80 m) cell,[38] and a more compact reflective helical cell,[39] which is capable of fitting into the spectrophotometer cell compartment.

Increasing automation makes possible the unattended mixing of the cell contents and cleaning of the cell from run to run. An electromagnetic stirrer was designed for optical cells.[40] A gas-driven stirrer motor was used to rotate very small magnetic stirring bars coated with Teflon in 1 mL cuvettes.[41] An automated sample cell cleaner that was based on one pump and two computer-controlled three-way valves was proposed.[42]

Some concern was given to the development of cells working in extreme conditions. A low-temperature (118 to 300 K) cell capable of withstanding pressures[38] up to 1 MPa and a high temperature (600 K) cell for pressures[43] up to 28 MPa were developed. Two cells capable of withstanding high hydrostatic pressures, up to 150 and 200 MPa, respectively, were described.[44,45]

For kinetic measurements, a cell was designed that allows separate reactants to temper-

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ature equilibrate in the cell compartment of the spectrophotometer before being rapidly mixed in a siphon mixing tube.[46] Electronic means of regulating the temperature with a Peltier device are replacing water jacketing. Microprocessors control the temperature programming while absorbance changes are measured.

Conventional cuvettes are gradually being replaced by flow-through cells, which became available in the field of spectrophotometry as a result of their application in chromatography and FIA. They enable both on-line detection and the rapid discrete sampling, purging, and rinsing of cells. Furthermore, a flow-through cell fixed in the sample compartment of a spectrophotometer virtually eliminates the errors originating from interchange and positioning of conventional cells.

The smaller sample amount used in highly efficient capillary liquid chromatography demands increased sensitivity of detectors. To achieve this, an approach involving the miniaturization of detection cells is the most popular. A detection cell based on ideas and components from advanced waveguide technology is gaining acceptance. Fiber optics can maximize optical pathlength for a constant cell volume, thereby increasing sensitivity without affecting the chromatographic information.[28] Requirements for flow-through detectors were discussed,[47] and a novel cell utilizing optical fibers to transmit light through a capillary was proposed. Theoretical considerations on the design of cylindrical flow cells utilizing optical fibers were made.[48] Some recent developments included a flow-cell suitable for on-column photometric detection[49] and a liquid-core, optical fiber total reflection cell for photometric detection in FIA.[50]

An increasing use of fiber optics for remote sensing, which eliminate the cuvette as such, was observed.[51,52] This is of interest, especially in process analysis, bioanalytical monitoring, and in field measurements where frequent *in situ* measurements are required or the access to the sample to be analyzed may be difficult for the analyst.

5. Detectors

Detection and data handling are those areas in spectrophotometry where the most significant progress has been made. The photomultiplier tube continues to enjoy high popularity in forward optics instruments, but its days are numbered. Although its poor sensitivity for higher wavelengths was overcome, the newer designs are expensive and not widely used. Competitive designs base on photosensitive silicon chips which are able to convert the light energy into an electrical signal. They offer many advantages, such as larger dynamic range, higher accuracy, higher speed, and smaller size, but their poor sensitivity in the UV region is still a drawback. A combination of a low-priced photomultiplier tube for wavelength range below 600 nm and a silicon photocell for the range above this value is the current choice. Because such a detector requires monochromatic light, the response at various wavelengths is measured at different times. This leads to poor efficiency and sometimes to poor accuracy.

In the past decade, detectors that permit the simultaneous acquisition of spectral information over the whole wavelength range have conquered the market. Simultaneous detection may be realized with a single detector that receives encoded information, which is later mathematically decoded (such as Fourier transformation), or by multichannel techniques in which spectral information is either spacially or temporally dispersed (optical imagers). The rapid development of the latter devices took place in the 1980s due to advances in semiconductor technology and the proliferation of powerful and relatively inexpensive microprocessor systems. Of the many optical image detectors, those gaining popularity in spectrophotometry include the silicon-intensified target tube (the vidicon), silicon photodiode array (PDA), and charge injection and charge transfer devices. Their principles of operation were discussed in a comparison paper.[53] The main drawback of vidicons and charge transfer devices is their poor sensitivity in the UV range. Furthermore, silicon vidicons are too slow for the detection of transient signals in HPLC and FIA. The best characteristics are shown by silicone PDAs, which, despite their relatively high price, have become the most widely used multichannel detectors in spectrophotometry. At present, they are supplied by most of the leading manufacturers of analytical instrumentation.

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The PDA detectors are simultaneous readout multichannel devices based on solid state optoelectronic image elements.[54–56] A PDA is an arrangement of *p*-type bars etched onto a *n*-type silicon chip. A series of reverse-biased *p-n* junction diodes (pixels) is thus formed. Each diode is fully charged before exposure to the light to be detected. The latter generates charge carriers in the silicon, which neutralize part of the stored charge proportionally to the intensity that is a function of the absorbance of the sample. The amount of charge lost is measured by measuring the current needed to recharge each diode in a defined time period. The recharging signal is thereafter amplified and digitized. The polychromatic light, after passing through the sample cell, is dispersed, usually by a fixed holographic grating, and projected onto the array so that each element receives the light of defined wavelength. To process the considerable amount of information acquired, an integrated microprocessor with a fast storing unit (usually a hard disk drive) and dedicated software is required. The advantages of PDA over conventional detectors were discussed in many papers.[53–61]

The most important feature of the PDAs is their ability to monitor all of the wavelengths simultaneously. Thus, a complete spectrum can be recorded within a few milliseconds because the scan time is not affected by the movement of the diffractor. This characteristic makes the optical system of PDA particularly interesting for detecting transient signals in hydrodynamic systems like chromatography and FIA.[55,59,61] In addition, because the PDA optics do not contain any moving parts, the wavelength reproducibility of the spectra obtained is very high and is limited only by the geometric constraints of the detector itself. Differentiation among the compounds whose absorption maxima are separated only by a few nanometers is thus possible. Furthermore, PDA systems offer greater sensitivity, dynamic range, and better tolerance to humidity, vibration, and electric and magnetic fields than conventional systems. One of the disadvantages, apart from the relatively high cost, includes a vulnerability to stray light effects because not all of the stray radiation inside the polychromator can be eliminated by a narrow bandpass filter, as it can in the single-wavelength, nonscanning spectrophotometer. The difficulties associated with wavelength calibration[62] and nonlinearity of UV calibration curves[63] were recently discussed. Precautions to be taken when designing data acquisition systems for use with PDA detectors were evaluated.[64]

Although PDAs show paramount superiority in hydrodynamic measurements, their potential for nonflow spectrophotometric multiwavelength analysis is also high. UV spectrophotometry with PDA detection will soon be the most frequently used technique for analysis of organic compounds that cannot be analyzed by gas chromatography. Future trends are aimed at developing a two-dimensional array and multifunctional instruments. An instrument using PDAs was shown to be capable of measuring absorbance and fluorescence or chemiluminescence simultaneously,[65] and 10-ns data collection was achieved with the introduction of diode array pulsed spectrometry.[66]

Applications of PDA detection in different areas of spectrophotometry are discussed later.

6. Data Acquisition, Handling, and Storage

The revolution in data-processing systems in the early 1980s also had a large impact on spectrophotometry.[67] It is not an exaggeration to say that the developments in this area have contributed to the renewed interest in spectrophotometry.

Whereas at the very beginning, simple dedicated computers were used for data collection, storage, and handling, now they constitute an integral part of the instrument itself. The incorporation of microprocessors in spectrophotometers has reached the point that now it is difficult to find an instrument without a microprocessor. In more instruments, even several microprocessors are present, each responsible for a different stage of analytical measurement. Their operation is controlled by a host computer supported by highly efficient data-processing peripherals and associated software. Currently, it is only the level

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of sophistication of the software that distinguishes the new instruments from their predecessors.

The computer is responsible for setting operating conditions: selecting a lamp, a filter, the wavelength drive, and data mode acquisition (absorbance, transmittance, or concentration), as well as controlling wavelength, autocalibration, and automated baseline storage and compensation. It also monitors the operation of the instrument during the run, carrying out self-tests, checking commands and data for conflicts, giving error messages when necessary, and often making suggestions about how to eliminate problems.

Apart from built-in units for complex data handling, many of today's spectrophotometers have the ability to interface with an external computer. The ability to handle data then becomes enormous. High-speed data processing together with large memory capacity provides the analyst with much greater data treatment capability leading to the development of new areas of application. The data manipulation package allows for graphical data display, spectrum smoothing, zooming, data and peak expansion, addition or subtraction of spectra, creating overlapped plots, obtaining hard copy from a peripheral printer or plotter, and programming complex data-handling operations such as rapid scanning, derivatization of spectra, and mathematic resolution of overlapping peaks by multiwavelength analysis. Analytical methods as well as acquired and/or edited data can be stored on diskettes or tapes and exchanged between laboratories.

The instrument control buttons were replaced by user-defined macroprograms, which are actuated from the keyboard by one key, mouse movement, or, in the future, by a vocal command. This has brought about the advent of a new generation of instruments termed "user-friendly". They are completely under the control of a microprocessor and in many cases the only thing they require is the sample. It must be emphasized, however, that even the most user-friendly instrument will not exempt the analyst from careful sampling, rigorous control of contamination, and critical evaluation of the results obtained.

B. Sample-Processing Analytical Techniques

1. Flow-Injection Analysis (FIA)

During the past decade, flow-injection spectrophotometric analysis has rapidly grown in popularity. This is because of its simplicity, versatility, precision, high sampling rate, low sample consumption, low cost, and ease of automation. In its classic form, this technique involves the injection of the sample into a stream of a chromogenic reagent, which transports it to the detector. The analysis requires a small amount of sample (10 to 300 μl), which, together with a light path of 10 mm usually used, is responsible for relatively high sensitivity. Virtually each color reaction in a homogeneous medium (provided it is sufficiently fast) can be adapted to flow injection processing producing a flow-injection spectrophotometric method. To have an advantage over conventional spectrophotometry, however, the time necessary for sample preparation should be no longer than that required for carrying out the color reaction.

FIA is particularly interesting when only a small amount of sample is available (physiological fluids), when the time necessary for the preparation of the sample is negligible (water, physiological fluids, or easily soluble pharmaceutical preparations), and when the number of samples to be analyzed is considerable. Hence, FIA has found many applications in environmental, clinical, pharmaceutical, and agricultural analysis where the need for automation is the greatest.

In the 1980s, over 600 papers on flow-injection spectrophotometry were published. An exhaustive bibliography up to 1985 was published by Růžička and Hansen[68] and is updated yearly by the Tecator Company. However, many methods were published without considering interferences. This makes them useless when facing a real analytical problem. Several modules for *on-line* sample handling were proposed that provide the opportunity not only for the separation of the analyte before mixing it with the chromogenic reagent but in some cases allow for its preconcentration thus giving lower detection

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limits.[69] These modules involve dialyzers,[70] ion-exchange[71] or redox microcolumns,[72] and liquid-liquid extractors,[73,74] and result in improvements of selectivity and sensitivity of FIA.

Interesting perspectives have opened for FIA with the advent of PDAs and their increasing availability. Theoretical aspects of the FIA-PDA association were discussed[75] a decade ago but it is only very recently that this union has gained noticeable momentum. Despite the increasing popularity of FIA and wider availability of PDAs, their combination is still hampered by the lack of commercial software. The software used by HPLC is too complex and hardly adaptable. Self-written programs must be used.

The major advantage of FIA-PDA for individual analyte determination is the ability to dramatically expand (by over three orders of magnitude) the range of the determination by using amplification and dilution methods. It is particularly important when the range of the analyte concentration in the sample is unknown. As the whole spectrum is recorded at the peak apex, either a less intensive wavelength or the sum of all may be chosen to fit the analyte concentration. Furthermore, because a large number of wavelengths are monitored simultaneously, the differences in spectral characteristics of the analytes are better exploited. FIA-PDA not only allows increasing the selectivity of a single determination, but also enables multicomponent analysis.[76] Some aspects are discussed later in the section devoted to advances in signal-processing analytical techniques.

Sensitivity in FIA with spectrophotometric detection is negatively affected by a difference in the refractive index between the sample and carrier solutions due to the so-called Schlieren effect.[77] This may be compensated for by a simultaneous measurement at another wavelength followed by subtraction of a nonspecific absorbance. This can easily be achieved with a PDA detector.[78]

Tables 1 and 2 show several flow-injection spectrophotometric methods used in inorganic and organic analysis, respectively. The selection of the methods was based on the criteria of little or no sample preparation and lack of interferences. A further restriction that had to be met to recommend a method was the proof of accuracy either by comparing it with an independent method of analysis or by analysis of certified reference materials.

2. Liquid Chromatography

Spectrophotometry is one of the most important detection techniques in liquid chromatography. In ion-chromatography, it continues to enjoy wide popularity for the direct determination both of anions and cations due to the large selection of post-column color reactions available. An extensive survey of the applications of spectrophotometry in ion-chromatography during the past decade can be found in Reference 148. The use of spectrophotometry as the detection technique in HPLC is equally important. Conventional methodology involves monitoring absorption at a fixed wavelength. This suffers from many limitations, e.g., when a sample contains several analytes, the trade-off wavelength is not optimal for any of them. Consequently, a loss of sensitivity for most of the detected compounds is observed. Moreover, because the detector does not discriminate between the analytes, any inefficiency in separation leads to errors in accuracy.

The advent of PDA detectors brought significant progress to the field of spectrophotometric detection in chromatography with respect to both sensitivity and selectivity. For each retention time value, the whole absorption spectrum is recorded so that the measurement is effectively made for every component at its absorption maximum. An increase in sensitivity in comparison with the fixed-wavelength detection is evident. In addition, depending on the concentration of the analyte, either a wavelength that is away from the absorption maximum may be chosen, or the response may be defined as the sum of signals at all the wavelengths monitored. Thus, the dynamic range of the determination may be extended, just as in FIA.

PDA detection also gives information on chromatographic peak purity and thus on efficiency of the separation, which is impossible to obtain with conventional detectors. Because the whole spectrum is monitored as a function of the retention time, the dependence of absorbance vs. retention time for a given substance should follow

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TABLE 1
Selected Applications of Spectrophotometry in Inorganic Flow Injection Analysis

Analyte	Color system	λ, nm	Range (detection limit), mg/l	Comments	Sample	Rate, h^{-1}	Ref.
Al	Eriochrome Cyanine R ascorbic acid		0.01–0.13% w/w	On-line electrochemical dissolution	Steels	20–40	79
NH_3	Glutamate dehydrogenase, NADH	340	0.05–0.6 *M* (0.005)	Enzymatic reactor in FIA system	Food Eye lotion	60	80
B	D-sorbitol/Methyl Orange	520	0.02–1.2		Seawater		81
	Azomethine-H		0.01–0.2 1–10	On-line preconcentration on a microcolumn	Tap water, natural waters	20 10	82
	Azomethine-H	420	Up to 4.0 (0.02)	Monosegmented flow analysis	Plants	120	83
	Azomethine	417	Up to 3.0	Stopped-flow	Plant leaves	60	84
BrO_3^-	KI/starch	570	0.5–5.0		Flour		85
Cd	Iodide/Malachite Green	685	0.1–3.0		Liquids from electrolysis plants manufacturing zinc	120	86
Ca, Mg	Murexide (Ca) Eriochrome Black T (Mg)	530	2–280 (total)	Simultaneous determination (water hardness)	Drinking water	50–55	87
	Glycol*bis*(2-aminoethyl-ether)-N,N,N′,N′-tetra-acetic acid/3,3′-*bis*(carboxymethyl)amino-methyl)-o-cresolphthalein	575	Up to 30	Simultaneous determination	Ground water River water Seawater	15	88
Ca + Mg Mg	1-(2-hydroxy-4-diethyl-amino-1-phenylazo)-2-hydroxynaphthalene-3,6-disulfonic acid Ba/EGTA (masking of Ca)	530	0.008–0.12 0.001–0.03 (0.6 μg/l, Mg) (5.0 μg/l, Ca)	Simultaneous determination, on-line preconcentration on a microcolumn	Chloralkali brines		89
Cr(VI)	1,5-Diphenylcarbazide		Up to 0.001 (0.5 ng)	Ion-exchanger phase absorptiometry	Natural water		90
Co	4-Phenylthiosemi-carbazone	430	0.4–14.4		Steels	48	91
Cu	4-(3,5-Dibromo-2-pyridy-lazo)-N-ethyl-N-(3-sulfopropyl)aniline	638	0.01–0.2		Serum	60	92
	Lead diethyldithiocarbamate	436	0.04–2.0		River water, plants	64	93
CN^-(total)	Isonicotinic acid, 3-methyl-1-phenyl-2-pyrazolin-5-one	548	Up to 1.0 (0.006)	On-line gas-diffusion separation/preconcentration	Waste water	40	94
Fe(III)	Thiocyanate	460	1–30	Stopped-flow	Wines	120	95
Fe(II) Fe(III)	Pyrocatechol Violet, cetyltrimethylammonium chloride	570	0.05–0.7	Kinetic simultaneous determination	Groundwaters		96
Fe(III)	Acetohydroxamic acid	440	0.5–10	Simultaneous determination	Extracts of hematite		97
Fe(II)	1,10-phenanthroline	512	10–60				
Fe(II) Fe(II) + Cu(II)	1-(2-Pyridylazo)-2-hydroxy-7-sulfonaphthalene	764 550	Up to 8×10^{-6} *M*	Simultaneous determination	Blood serum		98
Pb	Dicyclohexyl-18-crown-6 dithizone	512	0.05–2.0 (0.005)	On-line preconcentration on a microcolumn	Alloys Leachates Water	36	99
Mn	Tiron/H_2O_2		10^{-7} *M* range	Kinetic, catalytic	River water	40	100
Mo	Catalytic effect of Mo on the oxidation of I^- by H_2O_2		0.001–0.04	On-line removing of interferences on a microcolumn	Plants	40	101
Nd	PAN/Triton X-100	560	0.03–21	Micellar system	Glasses		102
NO_2^-	$TiCl_3$/sulphanilamide N-(1-naphthyl)ethylene diamine	530	0.02–5.0	On-line column elimination of Cu(II)	Potable waters	30	103
Pd	2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropyl-amino) aniline	612	0.01–0.1 (0.002)		Catalysts Dental alloys	50	104
PO_4^{3-}	Molybdate/vanadate	413	0.3–5.0		Biological tissues		105
P	Molybdate/$SnCl_2$	Red LED	Up to 0.1 (0.1 μg/l)	On-line preconcentration on a microcolumn	River water		106

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TABLE 1 (continued)
Selected Applications of Spectrophotometry in Inorganic Flow Injection Analysis

Analyte	Color system	λ, nm	Range (detection limit), mg/l	Comments	Sample	Rate, h^{-1}	Ref.
P(total)	Molybdate/Malachite Green/ thiosulfate		0–0.5 or 0.01–0.05	System includes a capillary digestor	Industrial waste water	30	107
P(dissolved organic)	Molybdate/$SnCl_2$	690	0.1–4.0 (0.01)	On-line photo-oxidation	Natural waters		108
K, Na	Benzo-18-crown-6 tetrabromophenolphthalein	620	0–2 × 10^{-4} *M* 0–2 × 10^{-3} *M*	On-line separation on a microcolumn	River water Tap water	15	109
SiO_4^{2-}	Molybdate/rodamine B	590	0.17–2.0		Tap, spring bottled water Waste water	40	110
Si	Molybdate/ascorbic acid	816	0–1.0 (0.03) (as SiO_2)	Phosphate interference is removed by heating to 80°C	Soil extracts	35	111
S^{2-}	2,2'-Dinitro-5,5'-dithiodibenzoic acid	500	0.1–20	Reagent injection	KCl brine		112
SO_4^{2-}	Fe(III)/$HClO_4$	355	10–150 25–600	Reagent injection	River water	20–30	113
SCN^-	5-Br-PADAP/dichromate	570	5–100 μ*M* (3.5 μ*M*)	Samples must be deproteinized	Saliva	60	114
Ti(IV)	H_2O_2	410	Up to 30		Brines	120	115
U	Arsenazo III Triton X-100	665	Up to 2.4 (6.6 μg/l)	On-line column reduction	Ore leachates		116
	Arsenazo III	649	Up to 0.3 (0.003)	On-line preconcentration on a microcolumn	Geological	30	117
V, Cr, hexacyanoferrate(III)	Leuco-Thionine Blue	670	(10^{-5} *M*)		Steels (Cr, V) Petroleum (V) Photographic solution (hexacyanoferrate)	110	118
H_2O	$SnCl_4$	305	3–150		Organic solvents	70	119
Zn	Zincon		Up to 2.0	On-line preconcentration on a microcolumn	Plant leaves	45	120
	Zincon	620	1–10 (0.05)		Waters, alloys Insulin formulations	80	121

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the same pattern for any wavelength. Poor separation can thus be detected. Moreover, it can be corrected by choosing another wavelength where the responses of unresolved components differ the most or by mathematic treatment of the data obtained.

The applications of PDA-HPLC have concentrated on industrial, clinical, and pharmaceutical chemistry and were reviewed.[61,149] A challenging field of applications is the determination of policyclic aromatic hydrocarbons (PAH), which can only be separated chromatographically to a certain degree. They were determined in synthetic samples[150,151] because real environmental samples are probably still too complex. Further improvement, however, is expected by using signal-processing techniques.[152]

3. Kinetic Methods

The number of papers devoted to kinetic spectrophotometric determination continues to be high, with a marked trend toward the application of methods development to real sample analyses. The methods focus on the determination of tran-

TABLE 2
Selected Applications of Spectrophotometry in Organic Flow Injection Analysis

Analyte	Color system	λ, nm	Range (detection limit), mg/l	Comments	Sample	Rate, h^{-1}	Ref.
Albumin	Bromocresol Purple		Up to 80 g/l	Controlled dispersion flow analysis	Human blood plasma	180	122
Amines (primary)	4-N-methylaminophenol dichromate	530	0.05–20		Drugs	120	123
Aryl-sulfoamines	N-(1-naphtyl)ethylene diamine	550	Up to 11 (0.2–0.5)		Drugs		124
Ascorbic acid	Iodide/iodate/starch or iodide/iodate	580 350	0.1–40		Fruit juice, vitamin C preparations	300	125
	Vanadotungstophosphoric acid	360	Up to 80		Tablets	80	126
	Chloramine T/KI/starch	650	15–150	Indirect determination	Urine	90	127
Berberine benzethonium	Tetrabromophenolphthalein/ethyl ester	610 610	10^{-6}–10^{-7} *M*				
	Tetrabromophenolphthalein/1,2-dichloroethane	610 610	10^{-6}–10^{-5} *M* 10^{-6}–10^{-5} *M*		Pharmaceuticals	45 30	128
Biuret	Copper sulfate	550	Up to 5000	On-line removal of NH_3	Fertilizers	50	129
Creatinine	Bromothymol Blue	Optosensing		On-line enzymatic	Serum	60	130
	1-Naphthol/biacetyl	520	Up to 250	Stopped flow	Meat		131
Enalapil	Bromothymol Blue	405	1.5–60		Tablets	80	132
Ethanol	NAD^+/alcohol dehydrogenase		0.002–0.016%	Simultaneous determination is possible	Wine, brandy	50–55	133
Ethanal	NAD+/aldehyde dehydrogenase		1.0–8.0				
Ethanol	NAD+/alcohol dehydrogenase semicarbazide	340	3–40	On-line enzymatic degradation and dialysis	Blood		134
Ethylenediamine	Pyridine-2-carbaldehyde/Cu(I)	475	1.4–85		Pharmaceutical	55	135
Formaldehyde	Pararosaniline/sulfite	570	2.0–50 0.2–10	Conventional FIA Stopped-flow FIA	Air		136
Glucose	Glucose oxidase/peroxidase/mutarotase/4-aminoantipyrine/phenol	535		Reversal flow analysis	Serum		137
	Bindschedler's Green	725	Up to 2.5 (0.02)	On-line enzymatic degradation to hydrogen peroxide	Blood plasma	20	138
Oxytetracycline	$Fe(III)/H_2SO_4$	435	10–80		Drugs	17	139
Pentachlorophenol	4-Aminoantipyrine/$Fe(CN)_6^{3-}$	637	1.0–60.0	Stopped-flow	Pharmaceuticals	28–48	140
Phenol	1-nitroso-2-naphthol/Ce(IV)		$1–8 \times 10^{-4}$ *M*	m- and p- substituted	Tablets	40	141
Promethazine	$Fe(III)/HClO_4$	515	6–117 (0.6)		Drugs		142
Chlorpromazine		535	6–124 (0.6)				
Thioproperazine		515	12–248 (1.3)				
Sucrose and total reducing sugar	Hexacyanoferrate(III) 1,10-Phenantroline/Fe(III)	512		Two signals are recorded per cycle	Sugar cane juice Molasses	40	143
Sucrose	Periodate/iodide			On-line inversion	Sugar cane juice Molasses	30	144
Tartaric acid	Vanadate	490	0.02–0.4	Degradation to ammonia	Wine	50	145
Urea	Enzymatic degradation to ammonia and hydrogen carbonate/optosensing of pH (reflectance spectrophotometry)		Up to 2.0 m*M*	Stopped-flow kinetic measurements for serum	Water Serum		146
	Biacetyl/thiosemicarbazide Fe(III)	530	0.5–15	Kinetic determination	Serum	20–30	147

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sition metals like copper, manganese, and iron and are based on their catalytic effect on indicator redox reactions. Relatively few methods for the determination of non-metals and organic compounds are published. An extensive survey of kinetic methods is published biennially in *Analytical Chemistry*.[153–157] In this review, the most interesting methods are discussed in sections devoted to the determination of individual species.

In general, kinetic spectrophotometric methods are characterized by high sensitivity but low selectivity so they often require a time-consuming sample preparation step. Catalyzed reactions in which the determined species is generated in another reaction are a noteworthy group. They combine the selectivity of the reaction that generates the species, which is actually to be determined, with high sensitivity of the catalytically induced indicator reaction. This involves mainly the methods based on the determination of hydrogen peroxide generated in the selective enzymatic reactions of many organic species.

The widespread use of microprocessor-controlled fast detectors has contributed to an increasing interest in kinetic measurements in hydrodynamic mode. This has resulted in the rapid development of the stopped-flow sample-processing technique for spectrophotometric analysis. In this technique, reactants are instantaneously mixed under high pressure, the resulting flow is abruptly stopped, and the reaction is monitored by measuring the changes in absorbance of the solution. The use of a fast detector is indispensable for studying the kinetics and mechanisms of fast reactions, but the stopped-flow methodology has also proved useful for quantitative analysis involving slower reactions because it minimizes reactant manipulation and affords higher precision than conventional kinetic approaches. The stopped-flow technique has numerous applications in both inorganic and organic spectrophotometric analyses due to high sample throughput, low sample consumption, and easy automation. It is well established in routine clinical, pharmaceutical, and environmental analysis. Applications of the stopped-flow spectrophotometric analysis were exhaustively reviewed.[158]

Until recently, the reaction progress was generally monitored at a single wavelength. The incorporation of fast-scanning detectors into stopped-flow systems has allowed an increase in the number and type of transient species determined. The prospects for stopped-flow multicomponent analysis were pointed out long ago,[159] but until the last decade no suitable detectors were commercially available. The growing use of spectrophotometers with PDA detectors offers a simple approach to the kinetic resolution of mixtures in routine analysis.[160] In some cases, a full UV/VIS spectrum can be obtained in as little as 2.7 ms, thus showing the great potential for stopped-flow measurements.[161]

Another possibility for multicomponent determination in kinetic spectrophotometric analysis is based on differential reaction-rate methods.[162] Diode-array detectors allow the kinetic determination of two or more species in a mixture by simultaneously monitoring the absorbance as a function of time and wavelength.

As often happens with batch methods, various catalytic reactions were examined and adapted to a flow-injection sample-processing mode. This trend seems to continue. However, in the case of kinetic methods, an intrinsic difficulty arises because of the continuous nature of the flow. It may be eliminated by the so-called stopped-flow FIA mode, which involves halting the flow at the detector and measuring the changes in the absorbance reflecting the evolution of the reaction during the stoppage. Nevertheless, the flow-injection mode does not allow fast reactions to be monitored and real time analysis is more difficult. The development of a stopped-flow continuous flow apparatus was proposed to overcome this drawback.[163] Some methods developed for kinetic FIA are mentioned in Tables 1 and 2.[84,95,96,100,131,136,140,146,147]

4. Solid-Phase Spectrophotometry

In recent years, the growing popularity of solid-phase spectrophotometry has been observed due to its simplicity, rapidity, ease of automation, and low detection limits achieved. These characteristics make it especially useful for the analysis of environmental waters. This technique is based on the measurement of the absorption of the color complex of the analyte sorbed on a solid

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support without subsequent stripping of the chromogenic species.[164-167]

Styrene and divinylbenzene solid supports are those most widely used. The solid support may act as a simple adsorbent toward the color complex of the analyte that was previously formed in solution, or may be modified with a complexing chromogenic reagent reacting with the species of interest. Another possibility is the sorption of all species on an unselective sorbent, e.g., a C18 column followed by selective derivatization of the analyte to form the color species.

The cell containing the sorbent is set in a spectrophotometer and the light transmitted is measured. In the solid phase, layer absorption and light scattering by the sample and the support contribute to the attenuation of the incident radiation intensity. The measurements are performed at the absorption maximum of the chromogenic species compared to another wavelength where only the solid support absorbs. The net absorbance is corrected for the blank, which was measured in the same way. These measurements may be carried out with the help of conventional spectrophotometers, resulting in low cost and easy availability. The only disadvantage is a difficulty in preparing a reference resin layer that has the same optical background as the color resin. This drawback may be circumvented by using derivative spectrophotometry,[168] which allows not only the background correction, but also significantly improves the sensitivity of the measurement.

Solid-phase spectrophotometry offers the advantage of *in situ* preconcentration of the analyte. Therefore, it is two to three orders of magnitude more sensitive than the corresponding conventional spectrophotometric methods provided that the analysis is performed with a 1-cm cell and 1 l of the sample.[167] Furthermore an increase in selectivity is observed due to different retention of the analyte and the interfering substances by the solid state support. In addition, the dynamic range of measurement is larger due to the possibility of the selection of a sample volume depending on the analyte concentration.

Integrated sorption and detection units based on solid-phase spectrophotometry have gained popularity in flow-injection sample processing.[169] The analyte is temporarily retained for detection on the sorbent placed in the flow cell and then immediately eluted. Solid-phase supports loaded with chromogenic reagents are also employed in sensors with fiber optics. In such a case, the intensity of the reflected rather than the transmitted light is measured. The problems associated with optosensing were reviewed.[170,171]

Applications of solid-phase spectrophotometry in analytical chemistry were exhaustively reviewed.[165-167]

C. Signal-Processing Analytical Techniques

A natural trend in spectrophotometry, as in other analytical techniques, is an approach to multicomponent analysis. This is of paramount practical importance especially in environmental, clinical, and pharmaceutical analysis. The quantitation of compounds with highly overlapping spectra in a mixture has always been a difficult analytical problem, especially at unequal analyte concentration levels. With the advent of fast-scanning PDA detectors and low-cost computers capable of processing complex data sets, new horizons have opened for mathematic processing of the information acquired. Two powerful signal-processing analytical techniques, multiwavelength and derivative spectrophotometry, rapidly developed. They not only offer many advantages regarding elimination of interferences and multicomponent determinations, but also contribute to more effective use of separation techniques.

1. Multiwavelength Simultaneous Analysis

Methods for multiwavelength analysis make use of different absorptions of analytes as a function of wavelength. In the ideal situation, each analytical wavelength gives a response characteristic of only one analyte. Simultaneous measurement then gives direct information on the concentration of the analytes; this rarely occurs in analytical practice. Dual wavelength spectrophotometry has long been popular because it eliminates the wavelength-independent matrix effect as well as analyzes two-component systems.

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It is, however, unreliable because two equations for two unknowns will yield the exact solution for the proportionality constants only when no interference occurs. This problem may be overcome by multiwavelength analysis, but its application on a larger scale was hampered until recently by the lack of a simple means of solving a set of equations with more than three unknowns. The advent of low-priced powerful microcomputers has enabled easy processing of spectrophotometric data. In addition, fast-scanning detectors have allowed elimination of errors resulting from poor time stability when a dual-wavelength method is used with a single wavelength spectrophotometer.

Modern multiwavelength analysis utilizes the reversed matrix representation of the Lambert-Beer law (Principal Component Analysis method [PCA]). It is applicable to the simultaneous determination of a larger number of components, even those with very close absorption maxima. Initially, multicomponent determinations were done by using a single wavelength for each component. The current approaches are based on multiple wavelength measurements and matrix least-squares data processing.

The most important factors in the least-squares analysis of spectrophotometric data are the selection of the wavelengths at which measurements are made and the extent of wavelength overdetermination that is necessary. In early papers, the wavelength selection for optimum precision and accuracy was mostly empirical and qualitative, while recently quantitative approaches based on statistical criteria have appeared. Computer selection is preferable, but requires the development of decision criteria. Some analytical wavelengths are better than others with respect to the accuracy and precision of the concentrations determined in the analysis. The various criteria and methods for the choice of the number and position of analytical wavelengths for quantitative analysis of multicomponent mixtures by the least-squares methods were discussed in many papers.[152,172–178]

General criteria for selecting analytical wavelengths for multicomponent mixtures by the PCA method require that, at the selected wavelength, Beer's law is obeyed and the absorbances are additive for each component. Furthermore, in an overlapping region, the selected wavelengths should be positioned at the absorption maxima of individual constituents to provide maximum sensitivity.

The same broad range of wavelengths is usually used to resolve each component in a mixture. However, it was demonstrated[152,174,176] that accuracy and precision may be improved if narrow wavelength ranges are chosen that highlight the spectrophotometric characteristics for each component of interest and deemphasize features of other potentially interfering components. The ability to improve results by this technique appears to depend on the difference between the absorbances of the analytes and potential interferents.

Selecting more analytical wavelengths can decrease the errors from any careless selection of correlated wavelengths. The number of wavelengths may be reduced when the absorptivities for each component at all selected wavelengths are neither equal nor proportional to each other. Potential improvements in precision when selecting a small number of optimal wavelengths were indicated.[173]

Calibration techniques that ensure high precision and accuracy with simultaneous low labor input (the minimum number of standards necessary) are important considerations. Improvements in data reduction schemes with the partial least-squares method and by application of fractional factorial designs for multivariate calibration were discussed.[179,180]

So far, most of the studies on multiwavelength analysis have focused on theoretical considerations. The *Chemical Abstract* search reveals a number of papers published regarding multiwavelength analysis, but these are seldom applied to the analysis of real samples. The accuracy of these methods for real samples is doubtful because of insufficient control of interferences and the lack of independent confirmation. Therefore, they seldom find their way to international journals. Some examples involve the determination of five hemoglobin derivatives,[181,182] determination of four active components in a commercial pharmaceutical formulation[173,179] and the determination of caffein, propyl phenazone, and phenacetin in their mixtures. In two-component systems, amodiaquine and primaquine[183] as well

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as *p*-aminosalicylic acid and *m*-aminophenol[184] were determined. Simultaneous determination of *o*-, *m*-, and *p*-cresols and α- and β-naphthols[185] as well as ethanol and ethanal[186] were made in flow-injection mode. In inorganic analysis, this technique was applied to the determination of niobium and tantalum as their ternary complexes with salicylfluorone and cetyltrimethylammonium (CTA).[177] Simultaneous determination of iron (II) and iron (III) with 1,10-phenanthroline and sulfosalicylic acid,[187] and zinc and nickel with 1-(2-pyridylazo)-2-naphtol (PAN)[188] were performed in flow injection mode.

The potential of a three-wavelength spectrophotometer with LEDs as the light source for simultaneous two- and three-component analysis was discussed.[189,190]

2. Derivative Spectrophotometry

Derivative spectrophotometry has enjoyed extensive activity during the last 10 years. Its dynamic development was due to the incorporation of electronic differentiators in most of the commercial spectrophotometers. These, together with associated software, are able to compute derivatives up to *n*-th order of the spectrum. Today, this technique has become a standard in spectrophotometric laboratories. It has proved to be very useful, providing both qualitative and quantitative information derived from mathematical processing of UV and VIS spectra. Since the principles of the derivative spectrophotometry were discussed, several reviews were published dealing both with the theoretical aspects[191–195] and practical problems.[196–201]

It must be emphasized that derivatization of spectra does not provide any additional information than that required during the measurement, but it allows for easier interpretation. This can best be seen in qualitative analysis. Positions of local maxima, however diffuse, may be precisely defined. Hence, any slight deformation of spectra due to the presence of some impurities can be identified. Compounds in which absorption maxima are too close to be separated by a conventional method can be resolved. In addition, when the order of the derivatives increases, more of the characteristic elements like maxima, minima, and zero-crossing points appear. They contribute to the enrichment of the description of the spectrum, thereby generating the possibility of obtaining a fingerprint for a given substrance.

In quantitative analysis, derivative spectrophotometry leads to an increase in selectivity and, in some cases, sensitivity of the determination due to elimination of errors resulting from overlapping bands. It permits measurements in turbid media (even in the UV range), thus eliminating the need for centrifugation and allowing *in situ* measurements using fiber optics. It has proved particularly useful in eliminating matrix interferences in the assay of many medicinal substances. Different sources of error in quantitative determinations were discussed.[191]

The possibility of resolving overlapping peaks makes derivative spectrophotometry a valuable tool for multicomponent analysis. Factors affecting selectivity in derivative spectrophotometry were evaluated.[191,202,203] The two factors having the greatest influence on the accuracy and precision of the results are the wavelength range and the derivative order. To date, the selection of both factors has been done empirically, in spite of the development of some theoretical methods to define this relationship quantitatively. An increase in the order of the derivatives makes the separation easier but decreases the sensitivity. A certain trade-off is thus necessary.

The potential of derivative spectrophotometry may be increased by acquiring the spectra to be derivatized with better accuracy and precision. The methodology based on consecutive absorbance-wavelength recordings is prone to errors due to imprecise grating positioning, which are amplified with increasing order of the derivatives. Moreover, the derivative spectra are somewhat delayed with respect to the original spectrum. Fast scanning possible with PDA and subsequent mathematical data processing eliminate this drawback and have facilitated the use of the derivative technique in a variety of applications.[204] Due to the high accuracy of PDA in spectra, aquisition mixtures of components yielding strongly overlapped spectra like polynuclear aromatic hydrocarbons[205] can be resolved.

Derivative spectrophotometry has numerous applications, mostly in pharmaceutical and clinical chemistry. These are based on the analysis

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of derivative spectra of the compounds to be determined in the UV region and were extensively reviewed.[200] Supplementary references can be found in the biennial reviews in *Analytical Chemistry*,[13-17] where a special section devoted to derivative spectrophotometry was introduced in 1984. In general, the accuracy of the methods published has not been confirmed by independent methods of analysis. It is thus prone to error, especially in analysis of more complex real samples of unknown composition, and must be evaluated carefully before the adaptation of a method to laboratory practice. Some procedures that are slowly becoming standards in clinical biochemistry involve the determination of porphyrins,[206,207] hemoglobin and its derivatives,[209-212] and phenylalanine[213] in blood, serum, urine, and amniotic fluid. Derivative spectrophotometry has proved useful for the simultaneous determination of two substances in biological fluids with very closely positioned absorption maxima, like bilirubin and hemoglobin,[214] carboxyhemoglobin and hemoglobin,[215] and especially in the determination of proteins. The references for the latter application may be found in previously cited papers.[198-200]

In inorganic analysis, two major areas of application can be distinguished. The first one involves the analysis of the absorption spectrum of analyte(s) without any or negligible chemical intervention. A classic example is the determination of rare earth elements (REE) in the presence of each other[216,218] and gadolinium in the presence of other REE.[219,220] Manganese was determined directly after oxidation to permanganate[221] in the presence of large amounts of nickel. Complexes with the inorganic ligands that were studied involved peroxocomplexes of titanium, vanadium, molybdenum,[222] and chloride complexes of palladium, platinum, and gold.[223] The second group involves chelation of the analytes with a sensitive but nonselective reagent followed by the analysis of the derivative spectrum of the mixture. Dithizone,[224-226] PAN,[227-231] and various hydrazones[232-237] were applied. The derivative spectrophotometric methods applied to inorganic analysis of real samples are summarized in Table 3.

D. Nonconventional Spectrophotometric Techniques

In conventional spectrophotometry, absorbance is a function of the measurement of the intensity of light transmitted through or reflected by the sample. The end of the 1970s brought a new generation of spectrophotometric methods that use an inherently different principle for measurement of the absorbance. These methods measure neither the transmitted nor reflected light but rather the power absorbed by the sample. Using absorbance measurement against a zero background, the deletion limits can be two to three orders of magnitude higher than that of conventional spectrophotometry.[241] Although this paper is generally meant to be restricted to classic spectrophotometry, to be thorough, we find it necessary to devote a small section to nonconventional methods.

The two most important and dynamic techniques are thermal lens and photoacoustic spectrophotometry. Both are based on the detection of the temperature change of the analyte solution induced by absorbed light. The development of these techniques was enabled only by a wider availability of cheaper and more reliable tunable lasers, which are the only light sources able to provide sufficient energy to induce a measurable temperature change.

In thermal lens spectrophotometry, the change in the refractive index resulting from an increase in temperature is measured. The principle of operation and applications of this technique were reviewed.[242,243] It is possible to measure very low absorptivities (down to 10^{-7}). Apart from its high sensitivity, other advantages of this technique are *in situ* capacity, precision, expanded dynamic range, and minimum sample requirements. The disadvantage is poor selectivity because absorption is measured at only one wavelength (the laser excitation wavelength) at a time. Furthermore, thermal lens spectrophotometry suffers from sensitivity losses in aqueous systems and requires recalibration for any significant change in solvent properties. Recently, some research has focused on dual[244-246] and multiwavelength analysis,[247] which are expected to increase considerably the

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TABLE 3
Selected Applications of Derivative Spectrophotometry in Analytical Chemistry

Analyte	Color system	Range, mg/l	Remarks	Sample	Ref.
Gadolinium	Direct determination	700–800	2nd order	In REE	218
			1st order	Nitrate salts	219
Iron	2-Pyridyl-3′-sulfophenyl methanone 2-pyrimidylhydrazone	μg/l range	2nd order	Tap and river water	234
	2-Pyridyl-3′-sulfophenyl methanone 2-pyrimidylhydrazone	0.010–0.210	4th order FIA system	Natural water	238
	2-Acetylpyridine 2-benzothiazolylhydrazone	0.6–13.0 μg	2nd order	Natural waters	236
	2,4,6-tri(2-pyridyl)-1,3,5-triazine	0.001–010	2nd order	Drinking water	238
Manganese	Oxidation to MnO_4^-	1×10^{-3}–2×10^{-5}%	4th order	Nickel salts	221
Mercury	PAN/CTA	Few	1st, 2nd order	Pesticides	231
	2-Pyridylketone 2-quinolylhydrazone	ng/ml	2nd order	Organomercurials	237
Molybdenum	Bromopyrogallol Red polyvinylpyrrolidine	d.l. 4 μg/l	3rd order	Drugs Multi-vitamin tablets	239
Neodymium	PAN/Triton 100	d.l. 11 μg/l	4th order	Glasses	229
Nickel	5-Methylfurfural-1-phtha lazinohydrazone		2nd order	Iron, steel, chemicals	232
	2,2′-Pyridyl-2-benzothiazolylhydrazone	d.l. 0.5 ng	2nd order	Iron, steel	233
Platinum	Iodide/dithizone	0.1–2	2nd order	Palladium salts	225
Rare earth elements	Direct determination		1–4th order, binary mixtures	Alloys	240

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selectivity of thermal lens spectrophotometry and enable multicomponent determinations.

Photoacoustic spectrophotometry uses acoustic detection of the thermally induced expansion of the illuminated sample. It is possible to measure the absorption spectra of solids, precipitates, sorbed complexes, and liquids. The principles and applications of photoacoustic spectrophotometry in gaseous and solid media were reviewed.[248] For the analysis of solids, the sample is placed in a closed cell containing air and a microphone. For liquids, the acoustic effect of thermally induced liquid expansion is measured by a piezoelectric crystal in the same medium.[249] Most recent developments involve portable open-ended photoacoustic cells for *in vitro* percutaneous spectrophotometric measurements.[250]

III. ADVANCES IN COLOR SYSTEMS

Although the number of papers abstracted in *Chemical Abstracts* on the developments of new spectrophotometric reagents and methods of analysis is still extremely large, a decreasing trend has been observed in the percentage of papers published in international analytical journals. This is because many of the new methods reported elsewhere are not competitive to the existing routine atomic spectrometric techniques. Furthermore, the point has been reached where any development in the field of organic reagents is usually restricted to a single analyte or a small group of them and cannot match the versatility of the progress made in instrumental techniques.

Well-known reagents continue to be revisited, and new application areas are sometimes

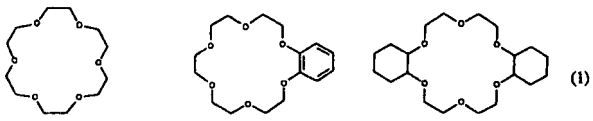
found for them. A general survey of organic analytical reagents[251] and reviews on well-established groups of compounds such as semicarbazones and thiosemicarbazones[252] and quinoxaline derivatives[253] have appeared. Simultaneously, many new reagents with superior performance to the existing ones, at least as claimed by the authors, have been synthesized. It must, however, be emphasized that it very seldom happens that a new reagent combines high sensitivity and selectivity, making it really worth recommending; crown ethers are the commendable exception.

The developments in new analytical systems have focused on five groups of compounds. They involve the already-mentioned crown ethers, the halogenated pyridylazo compounds, porphyrines, surfactants, and floatable ion-associates of anionic metal complexes with basic dyes. They are discussed here in separate sections. All of the other noteworthy reagents that have appeared during the last decade are discussed in sections devoted to individual elements.

A. Macrocyclic Compounds

Since the large-scale synthesis in 1967 of macrocyclic compounds, these compounds have become popular in different branches of chemistry, but their use as analytical reagents was rather rare until the late 1970s.[254]

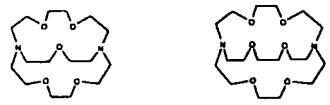
Analytically interesting macrocyclic compounds can be divided into crown ethers, aza, sulfa, oxa-aza, oxa-sulfa, and aza-sulfa crowns and cryptands. Crown ethers are compounds in which the ether oxygens are separated by 2 or 3 ($-CH_2-$) or (–CH=) groups. Some examples of structures are



(1)

18-Crown-6 Benzo-18-crown-6 Dicyclohexyl-18-crown-6

Aza, sulfa, and oxa-aza(sulfa) crowns have a structure like that of the crown ethers but with the oxygen atoms replaced with nitrogen (aza) or sulfur (sulfa) either completely or partially (oxa-aza, oxa-sulfa). These compounds are trivially denoted similar to crown ethers, e.g., 18-aza-crown-6. Cryptands can be described as three-dimensional oxa-aza crown ethers with the structure:



(2)

Cryptand 2.1.2 Cryptand 2.2.2

Macrocyclic compounds are characterized by the presence of a cavity capable of encapsulating a cation of defined size due to interactions with the electron-donating O, N, or S atoms binding to the cation. The complexes formed with cryptands are more stable due to the larger number of interacting atoms. Coordination chemistry of alkali and alkaline earth metals with macrocyclic compounds was discussed and the application in chemical analysis reviewed.[255,256]

Crown ethers and cryptands exhibit very selective extraction behavior, which makes them useful as analytical reagents. Two groups of attractive spectrophotometic systems can be distinguished with respect to the chromophore present in the system. It may be either the crown ether itself or an acid dye forming an ion pair with the positively charged crown ether metal complex. Extraction with crown ether derivatives is thus convenient to increase not only the selectivity, but also the sensitivity of the determination.

Appending a chromogen able to dissociate a proton to a crown ether ring offers attractive properties induced by the complexed cation. In a medium basic enough to dissociate a proton from the chromogen, the resulting anion interacts strongly with the crown-complexed cation compensating for the electric charge. The intramolecular ion pair formed under these conditions is hydrophobic enough to be extracted selectively from an aqueous into an organic phase. In addition, the formation of a zwitter-ion leads to considerable changes in its spectrum compared to that of the crown ether itself.

The chromogen side-arm of the crown ether may be incorporated either via a crown ring N atom[257–259] or via an azotype[260–265] or monobasic amine linkage[266–273] on the nitrogen or carbon atom of the crown ring. In the latter case, it is the amine proton that is dissociated off and the

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chromophore part may not contain the OH group. The monobasic amine linkage is advantageous due to the greater aqueous solubility of corresponding compounds and the significantly larger difference in wavelength between the absorption maxima of the protonated and deprotonated species. Although some authors regard the extractability to be independent of the type of the chromogen pendant involved,[260] others find that dramatically different metal selectivity can be obtained by changing the nature of the anionic sidearm on the same crown ether skeleton.[274]

The second category of methods is based on the formation of not intra- but intermolecular ion pairs. Initially, picric acid was used as the chromogenic anion,[275-279] but lately it has given way to more intensely colored acid dyes like some azo dyes,[280,281] Bromothymol Blue,[282,283] Bromocresol Green,[284] Eosine,[285-288] and Erythrosine.[289,290] The use of cryptands is relatively rare when compared to crown ethers. The use of cryptand 2.1.1. for the determination of sodium and cadmium[275,289] and cryptand 2.2.2. for the determination of lead[285,286] has been reported. Differences in the dissociation rate of cryptand 2.1.1. and 2.2.2. complexes with alkaline earth metals were utilized to develop kinetic methods of their determination.[291-293] Another possibility in this group of methods is for the extraction of the ion pair of the metal-crown ether complex associated with a nonchromogenic cationic partner followed by the reaction of the extract with a chromogenic reagent.[294-296]

Table 4 gives examples of the application of macrocyclic compounds in spectrophotometric analysis of real samples.

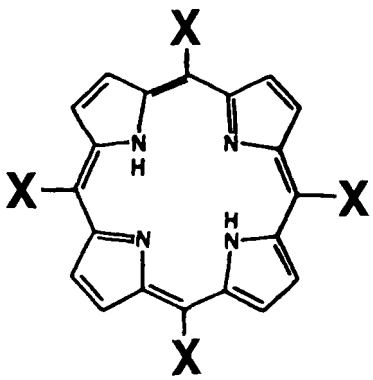
B. Halogenated Pyridylazo Compounds

Azo reagents play an important role in the spectrophotometric determination of metals. The principal azo reagents, PAN and 4-(2-pyridylazo)resorcinol (PAR), are among the most widely used reagents in analytical inorganic chemistry. In the 1970s, halogenated derivatives of pyridylazo compounds were reported to give much more sensitive reactions (a factor of 2 to 3) than the unsubstituted ones.[305,306] They have developed rapidly to allow for simple and very sensitive determination of most transition metals in aqueous or homogeneous aqueous organic media. The selectivity of these reactions may be controlled by the appropriate choice of the pH of the reaction medium as well as by masking the interfering species. The structures of compounds having the greatest analytical importance together with the abbreviations used throughout are given in Table 5.

Table 6 illustrates the applications of halogenated derivatives of pyridylazo reagents for photometric determination of elements.

C. Porphyrines

The role of metalloporphyrines in biochemistry has long been established, but it is only in the past decade that their importance in analytical chemistry was discovered. The attractiveness of porphyrines in this field is due to the extremely high molar absorption coefficients reaching a few hundred thousand liters per mole per centimeter at 400 to 500 nm (Soret band). The porphyrines skeleton and the most typical substituents are shown below:



(3)

X =	Phenyl-	1-Methyl-2-pyridyl-
	4-Carboxyphenyl-	1-Methyl-3-pyridyl-
	4-Sulfophenyl-	1-Methyl-4-pyridyl-
	4-Pyridyl-	N-Trimethyl-4-pyridyl-

The substitution of a methyl group for the hydrogen atom bound to nitrogen is possible but seldom occurs.

Two categories of methods may be distinguished in the spectrophotometric analysis with the use of porphyrines. In batch methods, the

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TABLE 4
Spectrophotometric Methods of Analysis with Use of Macrocyclic Compounds

Analyte	Color system	λ, nm	ε × 10^{-4} (μg/cm²)	Range	Sample	Ref.
Calcium	N,N′-bis(2-hydroxy-5-nitrobenzyl)-1,10-diaza-4,7,13,16-tetraoxacyclooctadecane	406	5.5	0.2–0.8	Serum	297
Copper and silver	1,4,8,11-Tetrathiacyclotetradecane/picrate	378 378	2.01	Up to 5.4	Steel	298
Lead	N,N′-bis(2-hydroxy-5-nitrobenzyl) cryptand-2.2.	406	4.47	10^{-6}–10^{-5} *M*	Zinc powder	258
	Dicyclohexyl-18-crown-6/dithizone	512		0.05–2.0	Alloys, sea-water, soil leachates	295[a] 296[a]
	Cryptand 2.2.2./Eosin		11.0	Up to 10^{-5} *M*		285
	Cryptand 2.2.2./Eosin	545		10^{-5}–10^{-4}%	Ag, Cd, Ni, Cu, Zn salts	286
	Hexaoxycycloazochromon		15.0		Waters	299
Lithium	Aza-12-crown-4	400		0.3–2	Serum, urine	257
	Aminobenzo-14-crown-4/picrate				Blood	269
	Dodecyl-14-crown-4 dinitrophenol	410			Serum	260
Potassium	4′-Picrylbenzo-18-crown-6	490		4–40	Portland cement	267
	4′-Picrylaminobenzo-15-crown-5	550		10–800	Seawater	300
	Dibenzo-18-crown-6/4-[(4-phenylamino) phenyl]azo-2,5-dichlorobenzenesulfonic acid	430		Up to 10^{-4} *M*	River water	280[a]
	Dibenzo-18-crown-6/4-[(4-diethylamino) phenyl]azo-2,5-dichlorobenzenesulfonic acid			Up to 10^{-4} *M*	River water	281[a]
	Dibenzo-18-crown-6/Bromothymol Blue	410	1.8	0.1–3.0	Fruit, beverages	283[a]
	Dimethylbenzo-18-crown-6/picrate			5–100 μg	Drilling water	301
	Dibenzo-18-crown-6/Bromocresol Green	410			Serum	284
Sodium	Cryptand 2.1.1./picrate	358	(0.017)	0–4 × 10^{-4} *M*	Serum	275
		522			Serum	302
	(2-Hydroxy-3,5-dinitrophenyl)oxymethyl-15-crown-5	423	1.40		Serum	303
Sodium and potassium	Benzo-18-crown-6/tetrabromophenolphthalein ethyl ester			Up to 2 × 10^{-3} *M* Up to 2 × 10^{-4} *M*		287[a]
Triethyl amine	Crowned 2,4.dinitrophenylazophenol/Ba(II)	520		0.2–4.0		304
Trimethyl amine	Crowned 2,4-dinitrophenylazophenol/Ba(II)	520		0.001–0.06	Fish	262[a]

[a] – in FIA system.

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TABLE 5
Halogenated Derivatives of Pyridylazo Compounds

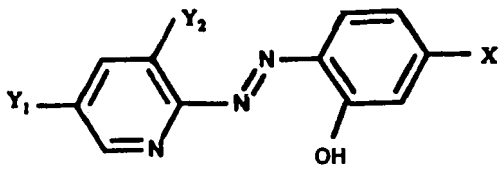
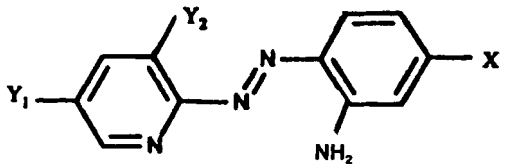
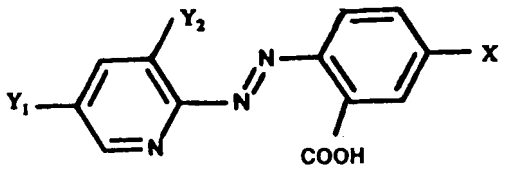
Compound	X	Y_1	Y_2
			
PAR	–OH	H	H
5-Br-PAR	–OH	Br	H
5-Br-PADAP	$-N(C_2H_5)_2$	Br	H
5-Cl-PADAP	$-N(C_2H_5)_2$	Cl	H
5-Cl-PADMAP	$-N(CH_3)_2$	Cl	H
3,5-diCl-PADMAP	$-N(CH_3)_2$	Cl	Cl
5-Br-PAPSAP	$-N(C_3H_7)(C_3H_6SO_3)$	Br	H
3,5-diBr-PADAP	$-N(C_2H_5)_2$	Br	Br
3,5-diBr-PAESPAP	$-N(C_2H_5)(C_3H_6SO_3)$	Br	Br
			
3,5-diBr-PADEA	$-N(C_2H_5)_2$	Br	Br
5-Br-PAPSA	$-N(C_3H_7)(C_3H_6SO_3)$	Br	H
3,5-diBr-PAESA	$-N(C_2H_5)(C_3H_6SO_3)$	Br	Br
			
3,5-diBr-PADEAB	$-N(C_2H_5)_2$	Br	Br
3,5-diBr-PADMAB	$-N(CH_3)_2$	Br	Br

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TABLE 5 (continued)

Note: PAR: 4-[(2-pyridyl)azo]resorcinol
5-Br-PADAP: 2-[(5-bromo-2-pyridyl)azo]diethylaminophenol
5-Cl-PADAP: 2-[(5-chloro-2-pyridyl)azo]diethylaminophenol
3,5-diBr-PADAP: 2-[(3,5-dibromo-2-pyridyl)azo]diethylaminophenol
5-Cl-PADMAP: 2-[(5-chloro-2-pyridyl)azo]-5-dimethylaminophenol
3,5-diCl-PADMAP: 2-[(3,5-dichloro-2-pyridyl)azo]-5-dimethylaminophenol
5-Br-PAPSAP: 2-[(5-bromo-2-pyridyl)azo](N-sulfopropyl)(N-propyl)aminophenol
3,5-diBr-PADAP: 2-[(3,5-dibromo-2-pyridyl)azo]diethylaminophenol
5-Br-PAESPAP: 2-[(5-bromo-2-pyridyl)azo](N-ethyl)(N-sulfopropyl)aminophenol
5-Br-PAR: 4-[(5-bromo-2-pyridyl)azo]resorcinol
3,5-diBr-PADEA: 2-[(3,5-dibromo-2-pyridyl)azo]diethylaminoaniline
5-Br-PAPSA: 2-[(5-bromo-2-pyridyl)azo](N-sulfopropyl)(N-propyl)aminoaniline
3,5-diBr-PAESA: 2-[(3,5-dibromo-2-pyridyl)azo](N-ethyl)(N-sulfopropyl)aminoaniline
3,5-diBr-PADEAB: 2-[(3,5-dibromo-2-pyridyl)azo]diethylaminobenzoic acid
3,5-diBr-PADMAB: 2-[(3,5-dibromo-2-pyridyl)azo]dimethylaminobenzoic acid

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analyte is allowed to react for a sufficiently long time with an appropriate porphyrine and the absorbance of the resulting complex is measured in aqueous solution or after extraction. This methodology, although hampered by kinetic factors, was used in the determination of copper,[341–343] cadmium,[344,345] and zinc.[346] The insufficient reaction rate was overcome in the determination of cobalt by using a cadmium-α,β,γ,δ-tetrakis(sulfophenyl)porphine (TSPP) complex instead of free porphine.[347] The reactions are usually very sensitive but not selective, and the analytes must be separated before determination. Selectivity may be achieved by taking advantage of the different stability of the analyte and the interferent complexes at different pH. Zinc could be determined in the presence of a large amount of cadmium.[348] Copper was determined in natural waters by solid-phase spectrophotometry after adsorbing the copper-α,β,γ,δ-tetrakis(4-N-methylpyridyl)porphine complex on a cation-exchanger and measuring the absorbance of the resin.[349]

The other group of methods makes use of the catalytic effect of the metal ion to be determined on the formation of the complex with α,β,γ,δ-tetrakis(4-sulfophenyl)porphine by another metal, usually manganese. Mercury was determined in this way after reduction with hydroxylamine, purging off, and absorbing by oxidation in permanganate solution.[350] The method was selective in the presence of cadmium, lead, and many other metals. Lead was selectively determined in the same way after extraction removal of iron with decanoic acid, which followed the reduction of mercury (II) and masking cadmium.[351] The same method applied to the determination of cadmium requires the coprecipitation of lead with manganese dioxide in the presence of sulfate and reduction of mercury (II).[352]

The method for kinetic determination of zinc in the presence of a large amount of cadmium makes use of the slower decomposition rate of the zinc-porphine complex in acid medium.[353] The extreme difference in the reaction of lead-TSPP and zinc-TSPP complexes with EDTA was

TABLE 6
Applications of Halogenated Derivatives of Pyridylazo Reagents for Photometric Determination of Elements

Analyte	Reagent	λ, nm	ε × 10^{-4}	Sample	Ref.
Antimony	5-Br-PADAP	610	5.9	Analytical reagents	307
	5-Br-PADAP	630	5.2	Waste water	308
Chromium	5-Br-PADAP	600	7.93	Waste water	309
	5-Br-PADAP	595	7.93	Alloy steel	310
Cobalt	5-Cl-PADAT	585	11.2	Steel, Ni metal	311
	3,5-diBr-PADMAP	673	11.5	Coal fly-ashes	312
	3,5-diCl-PADMAP	590	8.4	Mild steel	313
Copper	5-Br-PAR	510		Serum	314
	5-Br-PADAP	556	9.75	River water	315
	3,5-diBr-PAESA	638	12.4	Serum	316
Cyanide (indirect)	5-Br-PADAP	560		Waste water	317
Iron	3,5-diBr-PADMAP	615	9.4	Al alloys, well water Silicon carbide powder	318
	5-Br-PAR	510		Serum	314
	5-Br-PADAP	562	9.0		319
	3,5-diBr-PAESAP	568 (753)	8.8 (3.4)	Tap water	320
	3,5-diBr-PADEAB	624	11.0	Al alloys, tap water	321
Nickel	5-Br-PADAP	560	12.6	Al alloys, waste water	322
	3,5-diBr-PADMAP	618	14.5		
Palladium	5-Br-PADAP	620	4.5	Ti alloys	324
	5-Br-PAPSA	612	9.84	Dental alloys	325
Silver	3,5-diBr-PADAP	570	7.7	Waste water	326
	3,5-diBr-PADEAB	600	8.0	Waste fixing solutions	327
Titanium	5-Cl-PADAP	580	4.39	Optical glass, ceramic materials	328
	5-Cl-PADMAP	534	4.59	Ni alloys	329
Uranium	5-Br-PADAP	578	7.4	Leach liquor	330
	5-Br-PADAP	574	6.33	Phosphoric acid, rocks, gypsum, water	331
	5-Br-PADAP	579		Ore leachates	332
	5-Br-PADAP			Nuclear waste	333
	5-Br-PADAP	579	7.2	Natural, drinking, and waste water	
Vanadium	5-Br-PADAP	585	5.48	Al metal	335
	5-Cl-PADMAP	586	5.5	Steel	336
	3,5-diBr-PADMAP	640	5.95	Fuel oil, stack gas	337
Zinc	3,5-diBr-PADMAP	610	12.6	Coal fly-ashes	338
	5-Br-PAR	510		Serum	314
	3,5-diBr-PADAP	570	13.0	Waste water	339
Zirconium	5-Br-PADAP	585	15.4	Zircon	340

utilized for the determination of zinc in pure lead metal.[354,355] N-Methyl-porphyrines react faster than porphyrines and were utilized in a kinetic method for the differential determination of copper and zinc in serum.[356]

The inhibitory effect of cyanide on the reaction of some porphyrines was exploited for the indirect spectrophotometric determination of cyanide in waste water samples.[357]

The applications of porphyrines in spectrophotometric analysis are summarized in Table 7.

D. Surfactants

The use of micellar systems in spectrophotometry continues to be extensive and many new methods using surfactants were developed during

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TABLE 7
Spectrophotometric Methods with Use of Porphyrines

Analyte	Porphyrine	λ, nm	ε × 10^{-5} (ng/cm^{-2})[a]	Comments	Sample	Ref.
Cadmium	α,β,γ,δ-Tetrakis(1-methyl pyridinium-3-yl)porphine	441	3.61	Conventional and 2nd order derivative spectrophotometry		344
	α,β,γ,δ-Tetrakis(1-methyl pyridinium-4-yl)porphine	450	2.20	Conventional and 2nd order derivative spectrophotometry		344
	α,β,γ,δ-Tetrakis(4-N-trimethyl aminophenyl)porphine	433	5.8			345
	α,β,γ,δ-Tetrakis(4-sulfo phenyl)porphine	413	(0.143)	Kinetic; decrease in reagent absorbance is measured		352
Cobalt	α,β,γ,δ-Tetrakis(4-sulfo phenyl)porphine	432	2.4			347
Copper	α,β,γ,δ-Tetrakis(1-methyl pyridinium-3-yl)porphine	429	4.0			343
	α,β,γ,δ-Tetrakis(4-N-trimethyl aminophenyl)porphine	432	5.14			342
	α,β,γ,δ-Tetrakis(4-N-methyl pyridyl)porphine	424		Optosensing	Natural waters	349
	α,β,γ,δ-Tetrakis(1-methyl pyridinium-4-yl)porphine	423 (446)	2.3	Dual wavelength spectrophotometry	Seawater	341
Copper and zinc	N-Methyl-α,β,γ,δ-tetrakis (4-sulfonatophenyl)porphine	439 430		Kinetic	Serum	317
Iron (III)	5-(3,4-Dihydroxyphenyl)-10,15,20-triphenylporphine sulfonate	430 415	1.09 5.37	1:1 complex (pH 4) 1:2 complex (pH 7)		358
Lead	α,β,γ,δ-Tetrakis(p-sulfophenyl)porphine	413	2.75 (0.75)	Kinetic; decrease in reagent absorbance is measured	Rain water	351
Manganese	α,β,γ,δ-Tetrakis(4-carboxyphenyl)porphine	469	(0.56)			359
Mercury	α,β,γ,δ-Tetraphenylporphine sulfonate	413	(0.038)	Kinetic; decrease in reagent absorbance is measured		350
Zinc	α,β,γ,δ-Tetrakis(1-methyl pyridinium-3-yl)porphine	435	(0.186)		Tap water	346
	α,β,γ,δ-Tetrakis(4-sulfo phenylporphine	425	4.45			348
	α,β,γ,δ-Tetrakis(4-sulfo phenyl)porphine	421	2.7	Kinetic	Tap and river water, pure Cd metal	353
	α,β,γ,δ-Tetrakis(4-sulfo phenyl)porphine			Kinetic	Pure Pb metal	354
	α,β,γ,δ-Tetrakis(4-sulfo phenyl)porphine			Kinetic	Pure Pb metal	355
Cyanide	α,β,γ,δ-Tetrakis(1-methyl pyridinium-2-yl)porphine		(0.133)	Indirect	Waste water	357
	α,β,γ,δ-Tetrakis(1-methyl pyridinium-3-yl)porphine		(0.126)	Indirect		357
	α,β,γ,δ-Tetrakis(1-methyl pyridinium-4-yl)porphine		(0.234)	Indirect		357

[a] Sandell's sensitivity.

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past decade. Cationic surfactants are the most popular. When added to a solution containing a negatively charged colored binary complex, they lead to a new analytical system of enhanced sensitivity and batochromically shifted analytical wavelength.

The state-of-the-art knowledge on the mechanism of interactions in micellar systems was discussed,[360–364] but many questions still remain. Despite considerable attention paid to the nature and mechanism of these types of reactions, there is still no comprehensive theory that can explain all aspects of the formation of the ternary complexes when the cationic surfactant concentration is changing in the system.

Mechanistic studies are mostly done by spectrophotometry due to the wide availability of this technique. They result in the development of many methods based mostly on sensitization of reactions with triphenylmethane dyes. Apart from that, other chromogenic reagents involve azo compounds,[365–368] xanthene dyes,[369–373] oxine derivative,[374] dihydroxycarboxychromenol,[375] and phenylfluorone[376–380] and its derivatives.[381–387] The methods described are usually very sensitive ($\epsilon = 1–2 \times 10^5$), but the presence of all kinds of interferences makes them of limited usefulness in the analysis of real samples. Moreover, many of the methods developed are accompanied by high blank values due to absorbance of the chromogenic reagent-surfactant complex at the analyte wavelength. These restrictions reduce the potential of surfactants in spectrophotometric analysis. Table 8 surveys selected applications of micellar systems to the spectrophotometric analysis of real samples.

E. Flotable Ion-Associates with Basic Dyes

Basic nonchelating dyes enjoy high popularity in spectrophotometric analysis due to a high molecular absorption coefficient (about 1×10^5 l mol^{-1}cm^{-1}). They are able to form extractable ion-pairs with monovalent anionic complexes of metals leading to a variety of sensitive methods, which were discussed earlier.[1] Polyvalent anionic metal complexes also react with basic dyes, but the reaction products cannot be extracted by slightly polar solvents. Instead, the compounds formed accumulate during shaking on the phase boundary or on the wall of the separating funnel. The precipitate can be separated off and dissolved in a polar solvent, producing an intensely colored solution that forms the base of a flotation-spectrophotometric method of determination.

Until the early 1980s, flotation-spectrophotometric methods had only been known for nonmetals such as silicon, germanium, phosphorus (V), arsenic (V), and tellurium.[1] The last decade brought a rapid development of the methods based on this principle. This filled the gap existing in the lack of sensitive spectrophotometric methods for most of the platinum metals and some refractory metals (vanadium, niobium, and molybdenum).

Flotation-spectrophotometric methods for platinum metals were reviewed.[405,406] The floated compounds were found to be adducts composed of a simple ion-associate of the anionic complex of the metal with one to three molecules of basic dye and several molecules of the salt of the basic dye with a simple anion. The mechanism of this interaction has not been explained so far, but adducts must be considered as independent compounds since they are relatively stable and do not decompose when shaken with water. This fact enables selective washing out of the dye not bound to the metal being determined, eliminating or reducing considerably the blank value thus making these systems attractive for analytical chemistry. The most sensitive of these systems is based on an adduct of ten dye molecules with a simple ion-associate of gold bromide complex with Rhodamine B and it has the highest molar absorptivity ever obtained, 1.2×10^6 l mol^{-1}cm^{-1}.[407]

Flotation spectrophotometric methods developed in the past decade are listed in Table 9.

IV. ADVANCES IN PHOTOMETRIC METHODS FOR INDIVIDUAL ELEMENTS

A. Alkali and Alkaline Earth Elements

Until the last decade, sodium, potassium, rubidium, and cesium had not generally been de-

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TABLE 8
Spectrophotometric Methods of Analysis with the Use of Micellar Systems

Element	Chromogenic reagent	Surfactant	λ, nm	ε × 10^{-4}	Sample	Ref.
Aluminum	Chromazurol S	CP	640	12.5	Dialysis fluid	388
	Chromazurol S	CP	625	1.34	Tap water	389
	Eriochrocyanine R	CTA			Natural water	390
	Pyrocatechol Violet	Zephiramine	587	8.9	River water	391
	Chromazol KS	CP	625	10.2	Steel	367
	Chromazurol S	Zephiramine	640, 700		Tap water	392
	Chromal Blue G	CTA	660	16.2	Mg-based alloys	393
	Chrome Fast Pure Blue B	CTA	645	12.3	Steels, soils, plants	394
Cobalt	Phenylfluorone	CP	620	11.6	Soil	376
	5-Bromo-PADAP	Triton X-100	586	9.24	Potable waters	365
Fluoride	Th/Chromazurol S	CTA	635	11.0	Tap water	395
Germanium	Tetrabromosalicylfluorone	CP	520	13.0	Coal ash	382
	Phenylfluorone	HDP	530		Glasses	396
Iron	Chromazurol S	CTA	645	13.5	Analytical grade NaOH	397
	Chromazurol S	Zephiramine	630	16.0	Seignette salt	398
	Pyrocatechol Violet	CTA	605	13.5	Felspar, Portland cement, analytical grade NaOH	399
	Chromal Blue G	CTA	693	14.3	Mg-based alloy	399
Indium	Phenylfluorone	CP	570	11.2	Alkali metal halides	377
Magnesium	Calmagite	Empigen BB	655		Plasma	366, 400
Nickel	Phenylfluorone	CP	540	10.0	Alloys	378
	PAR	CDBA	510	6.8	Alloys	368
Rare earth elements	Arsenazo III	CTA	660		Apatites, RRE oxides concentrates	401
Tantalum	Salicylfluorone	CP	520	11.5	Ores	381
Tin	Dihydroxycarboxychromenol	CP	520–550	4.2–7.9	Alloys	375
Titanium	Chromazurol S	CTA	565	7.3	Technical grade Al metal	402
	Phenylfluorone	Triton X-100	540	16.3	Soils	403
Vanadium	Eriochromcyanine R	CTA	575	7.9	Steel	404
Zirconium	o-Nitrophenylfluorone	CTA	540		Glasses	385

Note: CP, cetylpyridinium; CTA, cetyltrimethylammonium; CDBA, cetyldibenzylamine; HDP, hexadecylpyridinium.

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termined spectrophotometrically. A rapid development in synthetic chemistry of macrocyclic compounds, however, brought a dramatic increase in the number of photometric methods developed for these elements, also for lithium and the alkaline earth metals. These methods offer considerable advantages over the previous procedures due to high selectivity and sensitivity. They were discussed in the section concerning macrocyclic compounds and are enumerated in Table 4.

Some other noteworthy methods were developed. A method for the determination of lithium in blood serum based on its complex with Thoron in alkaline acetone medium was proposed.[430] An indirect determination of potassium, rubidium, and cesium based on the reaction of their tetraphenylborates with mercury (II) chloranilate was described.[431] Addition of electrolytes at high concentrations was found to reduce the interferences and decrease the detection limit for berylium in a well-known method based on its complex with Chromazurol S.[432] Of ten reagents evaluated for the photometric determination of calcium,[433] Chlorophosphonazo III, Thymolphthalexon, and Arsenazo I were found to be the best. Chlorophosphonazo MA, I and III were used for determination of water-soluble calcium in soils; [434–436] the latter reagent was used also in a flow-injection mode.[437] Some new reagents used

TABLE 9
Selected Flotation-Spectrophotometric Methods of Analysis

Analyte	Anion (dye)	Flotation solvent (dissolution)	λ, nm	$\epsilon \times 10^{-5}$	Sample	Ref.
Gold	I^- (Methylene Blue)	Cyclohexane (methanol)	655	3.4	Blister copper	408
	Br^- (Rhodamine 6G)	DIPE (methanol)	545	11.6	Blister copper	407
Iridium	$SnCl_2$ (Rhodamine 6G)	DIPE (acetone)	530	3.6		409
Mercury	I^- (Methylene Blue)	Cyclohexane (methanol)	655	3.4	Cadmium metal	410
Molybdenum	3,5-dinitrocatechol (Rhodamine B)	Cyclohexane (acetone)	555	2.1	Plants	411
Niobium	3,5-dinitrocatechol oxalate (Rhodamine B)	Cyclohexane (acetone)	555	2.1	Geological materials	412
Osmium	Cl^- (Rhodamine 6G)	Toluene (acetone)	530	4.0	Platinum metals	413
	$SnCl_2$ (Rhodamine B)	Cyclohexane (acetone)	560	4.1	Platinum metals	414
	$SnCl_2$ (Rhodamine B)	Toluene (acetone)	560	6.2		415
	SCN^- (Methylene Blue)	Toluene (acetone)	655		Crucible platinum	416
	$SnCl_2$ (Crystal Violet)	Cyclohexane (75% acetone aq.)	600	2.0	Technical platinum	417
Palladium	Br^- (Rhodamine 6G)	Benzene (DMF)	530	3.0	Platinum	418
	$SnCl_2$ (Rhodamine 6G)	DIPE (acetone)	530	2.8	Silver metal	419
Platinum	$SnCl_2$ (Rhodamine 6G)	DIPE (acetone)	530	2.8	Palladium metal	420
Rhodium	$SnCl_2$ (Malachite Green)	DIPE (75% acetone aq.)	627	3.4		421
	$SnCl_2$ (Rhodamine 6G)	DIPE (acetone)	530	4.0	Palladium metal	422
Ruthenium	Cl^- (Rhodamine 6G)	Toluene (acetone)	530	5.1	Crucible platinum	423
	$SnCl_2$ (Rhodamine B)	Cyclohexane (acetone)	530	5.0	Crucible platinum	424
	$SnCl_2$ (Crystal Violet)	Toluene (acetone)	600	2.1		425
Ruthenium (osmium)	SCN^- (Capri Blue)	DIPE (methanol)	630	2.7		426
Silver	SCN^- (Rhodamine 6G)	DIPE (acetone)	530	1.5	River water	427
Tin (II)	$SnCl_4^-$ (Rhodamine 6G)	DIPE (acetone)	530	0.9	Analytical grade $SnCl_4$	428
Vanadium	3,5-Dinitrocatechol (Rhodamine B)	Cyclohexane (acetone)	555	2.1	Plants, vegetables	429

Note: DIPE, diisopropyl ether.

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for calcium determination included glyoxal bis(4-hydroxybezoylhydrazone),[438,439] 2-[2-(8-hydroxyquinolyl)azo]-1-naphthol,[440] Chlorindazon C ($\epsilon = 1.9 \times 10^4$ at 586 nm),[441] and 1,3-bis[2-pyridyl)methyleneamino]urea ($\epsilon = 1.6 \times 10^4$ at 430 nm).[442] The reaction of Calcichrome with calcium was reexamined ($\epsilon = 1.4 \times 10^4$ at 522 nm).[443] 2-(2-Hydroxy-3,6-disulfo-1-naphthylazo)-5-diethylaminophenol was synthesized and proposed for flow-injection determination of magnesium and calcium in waters.[444] The sensitivity of the Chromazurol S determination of calcium was increased by using a mixed ligand complex with 1,10-phenanthroline and cetylpyridinium (CP).[445]

B. Scandium, Lanthanides, Actinides

The development of derivative and multiwavelength spectrophotometry has increased the potential of many of the old methods by allowing the poor selectivity of sensitive reagents to be circumvented. These reagents, such as Arsenazo III, Chlorophosphonazo III, or Carboxynitrazo, are commonly used in the photometric determination of REE. A novel spectral subtraction technique was proposed for a mixture of eight REE. A narrow wavelength range, which contained the most characteristic absorption peak for each component of the mixture, was used for the simultaneous determination of a single or a few target

constituents.[446] Irrespective of the development of instrumental techniques, methods for the more efficient separation of REE from the matrix are necessary. Mixed ligand chelate extraction of lanthanides in systems involving 8-quinolinol and its derivatives,[447,448] substituted phosphinic acid,[449] and N-phenylbenzohydroxamic acid[450,451] was studied. Cerium subgroup REE were determined in the presence of yttrium-subgroup elements by a novel reagent, m-Nitrochlorophosphonazo.[452] On the contrary, p-nitrochlorophosphonazo was proposed for the determination of yttrium in the presence of cesium-subgroup elements.[453] Chlorophosphonazo III was applied to the determination of REE in organic extracts.[454] The determination of REE in geological samples was reviewed.[455] An interesting method was proposed for *on-line* determination of europium (III) after reduction to europium (II).[456] Yttrium may also be determined kinetically basing on its catalytic effect on the reduction of molybdate to molybdate blue by ferrous ammonium sulfate[457] or ethylhydrazine.[458]

Sensitivity of the determination of scandium was found to increase when mixed ligand complexes with monocarboxylic acids[459] or diantipyrilmethane[460] and chromogenic species like PAR[459] or triphenylmethane dyes[460] were used. The common Xylenol Orange method for photometric determination of scandium was used after its separation by precipitation with oxalic acid.[461] Scandium was extracted by a mixture of 2-ethylhexylphosphonic acids[462] or mesityl oxide[463] and determined by Chlorophosphonazo III and Arsenazo I, respectively. A new sensitive reagent for the spectrophotometric determination of scandium, p-Chlorophosphonazo, was synthesized ($\epsilon = 1.54 \times 10^5$ at 762 nm).[464]

The Arsenazo III method continues to be widely used for uranium determination. It was combined with the extraction of uranium with diantipyrilalkanes[465] or 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone[466] after enrichment on cellulose derivatives[467] or ion-exchange.[468,469] Methods for separation, preconcentration, and determination of uranium in natural waters were reviewed.[470] Uranium and thorium were determined sequentially with Arsenazo III after extraction with methylisobutylketone (MIBK) from acid-deficient aluminum nitrate medium[471] or ion-exchange.[472] Highly accurate determination of uranium in certified reference materials using Chlorophosphonazo III after the uranium was separated by ion-exchange was described.[473] A very sensitive method ($\epsilon = 3.56 \times 10^5$ at 600 nm) based on the ion-pair of uranium-thiocyanate complex with Rhodamine B that is stabilized with polyvinylalcohol was reported for the determination of uranium in seawater.[474]

Arsenazo III determination of thorium was the subject of four noteworthy papers,[475–478] one of which concerned highly accurate determination of thorium after ion-exchange separation.[478] *m*-Carboxychlorophosphonazo was proposed for the sensitive determination of thorium in thorium-tungsten alloys ($\epsilon = 1.03 \times 10^5$ at 676 nm).[479]

Methods for the determination of transplutonium elements were reviewed.[480]

C. Titanium, Zirconium, Hafnium

A series of sensitive methods based on ternary titanium complexes with different complexing agents (fluoride, hydrogen peroxide, hydroxylamine, and hydroxamic acid derivatives) and chromophoric species like Alizarine ($\epsilon = 7 \times 10^4$ at 513 nm),[481] o-hydroxyquinonephthalein ($\epsilon = 3.1 \times 10^5$ at 513 nm),[482] phenylfluorone ($\epsilon = 1.23 \times 10^5$ at 560 nm,[483] $\epsilon = 1.71 \times 10^5$ at 600 nm,[484] and $\epsilon = 2.33 \times 10^5$ at 540 nm)[485] were developed. Anionic titanium complexes with organic chelating agents like Pyrocatechol Violet,[486] catechol derivatives,[487] and p-chloromandelic acid[488] were found to be extractable as ion-pairs with tridodecylethylammonium bromide,[486] different basic dyes,[487] or Malachite Green[488] to give sensitive photometric methods ($\epsilon = 5 \times 10^4$ at 586 nm, $\epsilon = 1.5–2.0 \times 10^5$ at 640–655 nm, and $\epsilon = 1.3 \times 10^5$ at 630 nm, respectively). Titanium was also determined spectrophotometrically after ion-exchange separation with diantipyrilmethane (DAM)[489] and Tiron.[490]

Other reagents proposed for the determination of titanium involve 3-hydroxy-2-naphthaloylhydrazone[491] and Chlorophosphonazo I,[492] which was also used for zirconium and hafnium.[492] There is still very little discrimination between zirconium and hafnium in the spectro-

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photometric analysis. Zirconium and hafnium were determined in alloys after their separation by precipitation with *p*-bromomandelic acid with Xylenol Orange.[493] Methods for the determination of zirconium and hafnium in the presence of each other were reviewed.[494] Sensitive spectrophotometric determination of zirconium with F-Alizarine ($\epsilon = 1.52 \times 10^5$ at 556 nm) was reported.[495] Zirconium and hafnium were usually separated from each other by extraction[496–498] or ion-exchange,[499] followed by determination usually with Xylenol Orange or Arsenazo III.

D. Vanadium, Niobium, Tantalum

Photometric determination of vanadium is still realized mostly with azo compounds, especially PAR ($\epsilon = 5 \times 10^4$ at 560 nm). Selectivity of the determination is achieved either by ion-exchange[500–502] or extraction[503] separation. Other azo reagents: 5-(8-quinolylazo)-2-monoethylamino-p-cresol ($\epsilon = 4.2 \times 10^4$ at 530 nm)[504] and some halogenated pyridylazo reagents discussed in Table 6 were proposed.

Some methods to increase the sensitivity of vanadium determination by ion-pairing of basic dyes with anionic vanadium complexes were described. Vanadium 3,5-dinitrocatechol complex forms extractable or flotable ion-associates with Brilliant Green or Rhodamine B for sensitive vanadium determination ($\epsilon = 1.7 \times 10^5$ at 630 nm or 2.1×10^5 at 555 nm), respectively.[429,505] Similarly, molybdovanadophosphate forms an extractable ion-pair with Malachite Green ($\epsilon = 1.86 \times 10^5$ at 630 nm).[506] Color anionic complexes of vanadium with Pyrocatechol Violet or Bromopyrogallol Red were extracted as ion-pairs with tridodecylethylammonium and diphenylguanidine to give analytical methods of moderate sensitivity but increased selectivity ($\epsilon = 4$ or 7×10^4 at 579 or 580 nm, respectively).[507,508]

N-*m*-Tolyl-N-phenylhydroxylamine was proposed as an extraction-photometric reagent for the determination of vanadium in coal and coal fly-ash.[509] Other reagents developed for the vanadium determination include 5,5′-methylenedisalicylhydroxamic acid,[510] 5-methoxycarbonyl-2-pyridinehydroxamic acid,[511] and phenylfluorone ($\epsilon = 2.1 \times 10^5$ at 520 nm).[512] Salicylfluorone was reported to be a sensitive reagent for extraction spectrophotometric determination of vanadium ($\epsilon = 1.2 \times 10^5$)[513] and niobium ($\epsilon = 2.1 \times 10^5$ at 523 nm).[514] Anionic complexes of niobium with catechol derivatives were studied,[412,515] resulting in the development of an extraction-photometric method based on the ion-associate of the niobium complex with tetrabromocatechol and Brilliant Green ($\epsilon = 1.3 \times 10^5$ at 640 nm)[515] and a flotation-spectrophotometric method based on the 3,5-dinitrocatechol-Rhodamine B system ($\epsilon = 2.1 \times 10^5$ at 550 nm).[412] Moderately sensitive methods based on the extraction of thiocyanate niobium complexes with triphenylguanidine[516] and dibenzo-18-crown-6 ether[517] were reported. An extraction-spectrophotometric method for the determination of niobium with N-benzoyl-N-phenylhydroxylamine (BPHA) and PAR in nonaqueous media ($\epsilon = 3.35 \times 10^4$ at 547 nm) was developed.[518]

The well-known formation of intensely colored ion-associates of tantalum hexafluoride with basic dyes was revisited and used to develop methods for the determination of tantalum in the presence of niobium in alloys,[519] ores,[520,521] and mill products.[521] Salicylfluorones in the presence of surfactants were used for sensitive determination of tantalum methods ($\epsilon = 1.6–1.8 \times 10^5$ at 512 nm).[522,523] The ternary tantalum complex of 2-(2-thiazolylazo)-5-dimethylaminophenol and 1,3-diphenyl guanidine[524] was extracted to give a spectrophotometric method. Extraction-spectrophotometric determination of tantalum with substituted N-arylhydroxamic acids was discussed.[525]

Catalytic effect of vanadium on the oxidation of gallic acid by bromate[526,527] or persulfate,[528] pyrogallol, and chlorpromazine[529] by bromate was utilized to give methods with a very low absolute detection limit (about 0.5 ng). In an interesting method, the catalytic effect of vanadium on an oxidative coupling of N,N-dimethylaniline and 4-aminoantipyrine was exploited for a sensitive and selective determination of vanadium in natural waters.[530]

E. Chromium, Molybdenum, Tungsten

1,5-Diphenylcarbazide continues to be the most popularly used organic reagent for chro-

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mium. It was used for the determination of chromium (VI) in natural waters after preconcentration of chromium (VI) by sorption followed by stripping[531] or optosensing.[532] The same reagent made possible the photometric determination of chromium (VI) in the presence of large amounts of chromium (III)[533] in minerals. Interference from hydrogen peroxide in the diphenylcarbazide method was examined.[534] Several sequential on-line flow-injection systems for chromium (III) and chromium (VI) determination with 1,5-diphenylcarbazide were described.[535–539] Dichromate could be extracted with tetraphenylarsonium[540] and tetramethylene bis(triphenylphosphonium),[541] the latter in an on-line mode. Reaction of chromium (III) and chromium (VI) with flavones in micellar media was studied as the basis for photometric determination.[542] *o*-Nitrophenylfluorone in the presence of CTA was proposed for the sensitive determination of chromium(VI) ($\epsilon = 1.1 \times 10^5$ at 582 nm).[543]

Extraction separation and subsequent spectrophotometric determination of molybdenum and tungsten with various reagents were critically discussed.[544] The effects of solvent nature, reducing agent, acid, and amide on extractability and photometric characteristics were described for the spectrophotometric determination of molybdenum with thiocyanate and amide dimers of N-phenylacetamide.[545] The thiocyanate complex was used for the determination of molybdenum in steels (after extraction with ethylisobutrazine[546] or amidopyridine,[547] as well as in flow-injection mode[548]) and in seawater (after ion-exchange separation).[549] The thiocyanate complex of tungsten was used for its photometric determination in ore concentrates.[550] Extraction[551] and ion-exchange[552] were proposed to increase the selectivity of classic dithiol methods. 3,4-Dithiol was employed for rapid determination of tungsten in geological materials.[553]

A series of sensitive methods for molybdenum and tungsten based on extractable or flotable ion-associates of their thiocyanate,[554,555] p-chloromandelate,[556] bromopyrogallate,[557] and 3,5-dinitrocatecholate[411] complexes with basic dyes was developed. These methods are sensitive ($\epsilon = 1–3 \times 10^5$) but lack selectivity. Chelating dyes like Bromopyrogallol Red,[558–560] rutin,[561] dihydroxycarboxychromenol,[562] and Pyrocatechol Violet[563] formed the basis of photometric methods for molybdenum[558–560,562] and tungsten[560,561,563] ($\epsilon = 5–9 \times 10^4$).

Various trihydroxyfluorones were proposed for the determination of molybdenum or tungsten either in the presence of cationic surfactants[564–565] or as ternary mixed ligand complexes after extraction[566,567] to give $\epsilon = 1.0–1.3 \times 10^5$ or $\epsilon = 1.2 \times 10^5$ at 512 nm, respectively. The highest sensitivity was obtained for the determination of molybdenum with o-nitrophenylfluorone in the presence of CTA ($\epsilon = 1.55 \times 10^5$ at 530 nm).[564]

The catalytic effect of molybdenum on the oxidation of iodide by hydrogen peroxide[568] or rubeanic acid[569] was applied to sensitive determination of molybdenum in plants.

F. Manganese, Rhenium, Technetium

Spectrophotometric methods for the determination of manganese were recently reviewed.[570] A few new methods that gain selectivity based on the direct absorbance measurement of permanganate[571] or by its extraction as an ion-pair with ethylene bis(diphenylphosphonium) cation[572–573] showed poor detection limits. The formaldoxime method was applied to the determination of manganese in silicates.[574,575] Sensitive determination of manganese with Erythrosine[576] ($\epsilon = 1.05 \times 10^5$ at 544 nm) and a porphyrine[577] was proposed. A rapid spectrophotometric method using 4-(2-thiazolylazo)resorcinol (TAR) ($\epsilon = 4.2 \times 10^4$ at 540 nm) was developed.[578] Manganese was also determined by extraction-spectrophotometry with oxine and Aliquat 336,[579] 2,2′-dipyridyl and nitrophenylazocatechols,[580] and 1-(2-quinolylazo)-2,4,5-trihydroxybenzene in plants.[581] These methods are moderately sensitive ($\epsilon = 2–7 \times 10^4$), but relatively selective. The catalytic effect of manganese on the oxidation of Malachite Green,[582] N,N-diethylaniline,[583] p-fuchsin-leucobase,[584] sulfanilic acid,[585] Methylene Green,[586] as well as 3-(2-hydroxyphenylazo)pyridine-2,6-diol by hydrogen peroxide[587] was used to develop methods for the determination of manganese in chemical reagents[582,584] and water, milk, and

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beer.[586] A method based on the catalytic effect of manganese on autooxidation of succinimide dioxime was also reported for its determination in analytical grade reagents.[588]

Rhenium was predominantly determined by extraction of an ion-pair of perrhenate (ReO_4^-) with basic dyes; [589–592] some new ones like Astra Phloxine[591] or Astrazone Blue G[592] were proposed. Improvement in the sensitivity in photometric determination of rhenium after extraction of perrhenate with Nitrotetrazolium Blue was reported.[593]

Catalytic effect of copper (II) on the reduction of technetium by Variamine Blue was utilized in the kinetic determination of the latter element in nuclear fuels. Technetium itself was found to catalyze the reduction of Methylene Blue by tin (II) which was used to develop a method for synthetic nuclear fuels and waters.[595] The catalytic effect of technetium (VII) on the reduction of tetrahydroxy-1,4-bezoquinone by tin (II) was applied to the determination of technetium in plants.[596]

G. Iron, Cobalt, Nickel

A plethora of papers has continuously been published on new methods for the determination of iron. Most, however, show no significant improvement over the highly recommended 1,10-phenanthroline method.

An interesting group of methods based on extraction of ternary 2,2′-dipyridyl[597] or substituted triazines[598,599] iron (II) complexes with tetraphenylborate into molten naphthalene[597,598] or their sorption on microcrystalline naphthalene[599] was developed to increase the preconcentration factor and the selectivity of the determination. Iron (II) phenanthroline[600] and substituted triazine[601] complexes, which were associated with picrate, give sensitive and selective methods ($\epsilon = 1.3 \times 10^5$ at 510 nm and $\epsilon = 2.2 \times 10^5$ at 593 nm, respectively).[600,601] The iron (II) complex with 2-nitroso-1-naphthol-4-sulfonic acid was extracted with an azo pyridinium compound to give a molar absorption coefficient of 2.1×10^5 at 555 nm.[602]

Morin and phenylfluorone complexes of iron (III) were sensitized by Triton X-100 ($\epsilon = 6.15 \times 10^4$ at 618 nm)[603] and phenylfluorone ($\epsilon = 1.2 \times 10^5$ at 618 nm).[604]

Bis(2-pyridyl) -N,N,-bis[(8 -quinolyl)amino] methane ($\epsilon = 1.7 \times 10^5$ at 693 nm) was reported for the sensitive determination of iron in blood serum.[605]

The catalytic effect of iron on the oxidation of chromotropic acid,[606,607] N-methyl-p-aminophenol,[608] N,N-dimethyl-*p*-phenylenediamine,[609] and 3,5-diaminebenzoic acid[610] by hydrogen peroxide was exploited in selective, precise, and sensitive (1 to 10 ng/ml) methods for iron determination. Other kinetic methods utilized the catalytic effect of iron on the oxidation of sulfanilic acid[611] and α-hydroxy acids[612] by periodate, o-toluidine by chlorite,[613] and iodide by bromate[614] in fresh water.

2-(2-Benzothiazolylazo)-5-dimethylaminobenzoic acid was synthesized and applied to the sensitive extraction-spectrophotometric determination of cobalt[615] and nickel[616] in fuels ($\epsilon = 1.2 \times 10^5$ at 705 and 635 nm, respectively). 2-(2-Thiazolylazo)-4-methyl-5- (sulfomethylamino) benzoic acid was proposed for the sensitive photometric determination of cobalt in steel ($\epsilon = 1.13 \times 10^5$ at 655 nm).[617,618] A specific and sensitive photometric determination of cobalt with 3-(2′-thiazolylazo)-2,6-diaminotoluene was reported ($\epsilon = 9.7 \times 10^4$ at 590 nm).[619] Some new reagents for nickel(II) determination included 6-(5-methyl-1,3,4-thiadiazol-2-ylazo)-3,4-dimethylphenol,[620] 2-(2-thiazolylazo)- and 2-(2-benzothiazolylazo)-5-(dimethylamino)benzoic acids,[621] and 1-(2-pyridylazo)-2-naphthol-6-sulfonic acid.[622]

The ion association of the nickel phenanthroline and PDT complexes with tetraiodofluorescein[623] and ethyltetrabromophenolphthalein[624] resulted in two sensitive ($\epsilon = 1.9 \times 10^5$ at 556 nm and $\epsilon = 2.2 \times 10^5$ at 610 nm) and selective (in the presence of masking agents) extraction-photometric methods for nickel determination. Ion pairing and extraction of the cobalt tetrathiocyanate complex with Basic Turquoise,[625] Brilliant Green,[626] and tetramethylene bis(triphenylphosphonium)[627] improved the selectivity, but the sensitivity could not match that for nickel. 2-Nitroso-1-naphthol-4-sulfonic acid-cobalt complex was extracted as an ion-pair with tetrabutylammonium[628] and azo dye.[629] In the lat-

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ter case, good sensitivity ($\epsilon = 1.7 \times 10^5$ at 566 nm) was obtained.

Substituted hydrazones of 2-pyridinecarbaldehyde,[630] 3-pyridyl-3-sulfophenylmethanone,[631] and thiazole-2-carbaldehyde[632] were proposed as reagents for nickel ($\epsilon = 7.2 \times 10^4$ at 522 nm)[632] and cobalt ($\epsilon = 5.7 \times 10^4$ at 496 nm)[635] determination. Carboxybenzene S was applied to the sensitive ($\epsilon = 1.5 \times 10^5$ at 720 nm) determination of nickel in meteorites after separating the nickel with dimethylglyoxime.[633]

Very low detection limits can be obtained when exploiting catalytic effect of cobalt on the oxidation of Tiron (limit of detection [d.l.] 0.05 ng/ml),[634] protocatechuic acid (d.l. 0.005 ng/ml, FIA mode),[635] catechol,[636] and Pyrogallol Red.[637,638]

H. Platinum Metals

The largest group of methods developed for platinum metals in the past decade were based on the flotation of ion-associates of the platinum metals complexes with basic dyes.[405,406] Determination of this group of elements was reviewed.[639]

Dithizone extraction was revisited for the separation and determination of palladium and platinum.[640–642] The extraction-spectrophotometric method with dithizone was also reported for rhodium and iridium at elevated temperature,[643] but the sensitivity obtained was moderate.

Several methods were developed that are based on the extraction or the stabilization by gelatine of ion-associates of platinum metals complexes with Rhodamine B,[644–646] with Brilliant Green (osmium, $\epsilon = 2 \times 10^5$ at 640 nm),[647] Malachite Green (platinum, palladium),[648] and Methyl Green (platinum, $\epsilon = 1.45 \times 10^5$ at 635 nm). However, the sensitivity of these methods is inferior to the flotation-spectrophotometric methods. For rutenium, a method based on the ion-associate of the cationic rutenium(II)-PDT complex with tetrabromophenolphthalein ethyl ester was reported.[650]

The potential utility of 5-azo derivatives of rhodanine and its analogs for the spectrophotometric determination of noble metals was discussed.[651]

An interesting procedure was reported for determining the platinum group metals using successive extraction and analysis. First, di(*o*-tolyl)thiourea was used for the separation and determination of palladium (410 nm), then zinc dithizonate was used for platinum (730 nm). After reduction with stannous chloride, di(*o*-tolyl)thiourea was used for rhodium (410 nm), and an extraction with mercaptobenzothiazole for iridium (410 nm).[652] 2-Thiobarbituric acid was reported for the determination of rhodium[653] and palladium.[654] Other interesting reagents proposed for palladium include 5-phenylazo-8-aminoquinoline,[655] N,N-dimethyl-N′-(4-p-nitrophenyl-5-nitro-2-thiazolyl)thiourea,[656] bis(2-ethylhexyl)dithiophosphate,[657] and Arsenazo III after extraction from nitric acid medium.[658] Rhodium was determined by pyrazolone-(4-azo-2)-1-naphthol-4-sulfonic acid.[659] 1,2,3-Indanetrione monothiosemicarbazone was proposed as a reagent for osmium.[660]

Some oximes were proposed for extraction-photometric determination of iridium, rutenium, osmium, and palladium. These are α-benzylmonoxime[661] for iridium, α,α′-furyldioxime[662] for rutenium, phenanthrenequinone monoxime for iridium and osmium[663] and 4-salicylamido-1-diacetyl monoxime 3-thiosemicarbazone for palladium.[664]

Rutenium can be determined very sensitively (d.l. <1 ng/ml) and relatively selectively by a kinetic method using its catalytic effect on the oxidation of diphenylamine,[665] Tropaeolin 00,[666] *p*-ethoxychrysoidine,[667] Methyl Orange,[668] and Carboxyarsenazo[669] by periodate. A very sensitive method based on the catalytic effect of rutenium on the decomposition of the cobalt (III)-porphyrine complex by bromate[670] was reported.

I. Copper, Silver, Gold

The methods for this group of elements involving crown ethers, porphyrines, and azo compounds were discussed in previous sections. Of other reagents, Michler's thioketone was proposed for the sensitive ($\epsilon = 1 \times 10^5$) determination of copper,[671] gold,[672] and silver (in the presence of anionic surfactants).[673] Antipyrine

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dyes for extraction-photometric determination of gold were discussed[674] and Chrompyrazol I was applied to the extraction-spectrophotometric determination of gold.[675] Imidazolylisoquinolines were suggested as new sensitive and selective reagents for copper.[676]

The halogenide complexes of copper (chloride),[677] gold (bromide),[407] copper and gold (iodide),[408,678] and silver and gold (cyanide[679,680] and thiocyanide[427,681]) form ion-associates with basic dyes, which were proposed for sensitive determination of these metals ($\epsilon > 1 \times 10^5$) either after extraction[677–681] or flotation.[407,408,427] An ion-associate of the cationic 2,2′-biquinolyl copper complex with ethyl ester of Eosin (tetrabromophenolphthalein ethyl ester) formed the basis of a sensitive spectrophotometric determination of copper ($\epsilon = 9.4 \times 10^4$ at 610 nm).[682]

Other sensitive reagents examined include 1-phenyl-3-thiobenzoylthiocarbamide for copper ($\epsilon = 1.3 \times 10^5$ at 360 nm),[683] Cadion 2B in the presence of Triton X-100 for silver ($\epsilon = 1 \times 10^5$ at 565 nm),[684] and 4-(p-nitrophenylazo)-2-amino-3-pyridinol for silver ($\epsilon = 1.1 \times 10^5$ at 605 nm).[685]

Kinetic determinations of copper utilized its catalytic effect on the oxidation of thiosulfate by iron (III) (biological samples),[686] oxidation of chromotropic acid by hydrogen peroxide,[687] and aerial oxidation of hydroxyloamine (natural waters) (d.l. 0.6 ng/ml).[688] The catalytic effect of silver[689,690] and gold[691] on the oxidation of ethylenediamine,[689] antipyrine-8′-hydroxyquinoline,[690] and Variamine Blue[691] by persulfate was employed for the determination of these metals. Highly selective determination of gold was based on its catalytic effect on aerial oxidation of Variamine Blue (d.l. 2 ng/ml).[692] Silver was found to catalyze the reaction of hexacyanoferrate(II) with isonicotinoylhydrazide.[693]

J. Zinc, Cadmium, and Mercury

The methods for these metals with the use of pyridylazo derivatives, porphyrines, and crown ethers were discussed in the respective sections.

Dithizone is still the most widely used reagent for this group of metals. It was applied in FIA mode[694,695] for the determination of cadmium. Mercury and cadmium were determined as dithizonates in aqueous phase in the presence of Triton X-100[696] and methyltrioctylammonium,[697] respectively.

Many reports describing the synthesis of new hydrazones and semicarbazones and their application to the spectrophotometric determination of zinc and cadmium were published. They offer, however, no significant progress with respect to either sensitivity or selectivity in comparison with the dithizone method. Extraction-photometric determination of cadmium with diphenylcarbazone in the presence of 1,10-phenanthroline was a sensitive and selective method ($\epsilon = 9.4 \times 10^4$ at 536 nm) for cadmium.[698]

o-Hydroxybenzenediazoaminoazobenzene,[699] Cadion,[700] and Cadion 2B[701] in the presence of Triton X-100 were used for the sensitive ($\epsilon = 1–2 \times 10^5$) determination of cadmium[699,700] and zinc.[701] 1-(2′,3′-Dihydroxypyridyl-4′-azo)benzene-4-sulfonic acid[702] was proposed for the sensitive ($\epsilon = 1.3 \times 10^5$) determination of zinc. 2-(2-Thienyl)benzothiazoline was reported for the sensitive mercury determination in environmental samples ($\epsilon = 1.7 \times 10^5$ at 375 nm).[703] The reaction of mercury with thio-Michler ketone was investigated.[704] Trihydroxyphenylfluorones reacted with zinc, but the sensitivity obtained was rather moderate. 1-(1-Phthalazinyl-3,5-diphenylformazan was found to be the best of five substituted phthalazinylformazans for determining mercury in waters and copper-zinc ores ($\epsilon = 6.4 \times 10^4$ at 520 nm).[705] N-Substituted 4,6-diphenylpyridine-2-thiones for the determination of mercury in pharmaceuticals (314 nm) were studied.[706]

Ion-associates of thiocyanate complexes of zinc[707] and mercury[708] with basic dyes or ion-associates of iodide complexes of cadmium[709–710] and mercury[410,711] with basic dyes were the basis of sensitive methods for analyzing these metals in aqueous solutions stabilized by gelatine,[707–710] after extraction[711] or flotation.[410] Two of these methods showed very good sensitivity: $\epsilon = 4.2 \times 10^5$ for cadmium[709] and 3.4×10^5 for mercury.[410] Acid dyes Bengal Extra[712] and Eosine[713] were found to form intensely colored ion-associates with cationic complexes of zinc with 2,2′-

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bipyridine and zinc, cadmium, and mercury with phenanthroline, respectively.

K. Aluminum, Galium, Indium, and Thalium

Spectrophotometric methods for aluminum based on chelating dyes in the presence of surfactants are discussed in Table 8. Trends toward automation and increasing selectivity by extraction have been observed. Pyrocatechol Violet, Aluminon, and Eriochromcyanine R were compared for the determination of aluminum in an FIA system.[714] Pyrocatechol Violet was found to be the best.[715] Aluminum was also determined in an automated system by Xylenol Orange.[716,717] Extraction of ion-pairs of ternary complexes with anionic triphenylmethane dyes and suitable cations was discussed. The proposed systems involved: aluminum (indium)-Eriochrome Black T,[718] galium-Pyrocatechol Violet-tridodecylethylammonium,[719] or aluminum-Pyrocatechol Violet-Zephiramine ($\epsilon = 9.8 \times 10^4$ at 590 nm)[720] and aluminum-Bromopyrogallol Red-quaternary ammonium salts.[721]

Sulfonated azo dyes continue to enjoy high popularity. The metal/ligand ratios, formation constants, molar absorptivities, and potential interferences of several indium (III) complexes of sulfonated azo dyes were reported.[722] Color reactions between indium,[723] galium,[724,725] and 2,7-bisazosubstituted chromotropic acid derivatives in organic-aqueous media were studied and Picramine M was the reagent of choice. Complexation of aluminum, galium, and indium with Lumogallion was investigated.[726] Sulfonitrazo DAF was proposed for the determination of aluminum in waters.[727] A sensitive method for the determination of traces of thorium with substituted azooxinesulfonic acid in the presence of CP ($\epsilon = 1.3 \times 10^5$ at 560 nm) was proposed.[728] From the other azo reagents, antipyrylazo compounds were discussed for the determination of galium.[729]

Salicyloylhydrazone of pyridine-2-aldehyde and pyridoxal were studied as reagents for the determination of aluminum ions.[730] 1-(2-Pyridylmethylideneamine)-3-(salicylideneamine)thiourea[731] and a tris-substituted triazine were proposed for the sensitive determination of indium ($\epsilon = 8.4 \times 10^4$ at 530 nm).[732] Hematocyclin was proposed for the sensitive determination of aluminum ($\epsilon = 1.7 \times 10^5$),[733] and galium and indium ($\epsilon = 1.4 \times 10^5$).[734]

The most popular methods for the thallium determination involve the extraction of its bromide complex with basic dyes such as Rhodamine B,[735] Victoria Blue B,[736] and Astrazone Red GB.[737] The ion-associate of the galium chloride complex with Rhodamine B[738] was extracted, but better sensitivity could be obtained by extraction of the ion associates of galium catechol derivative complex with Brilliant Green ($\epsilon = 2 \times 10^5$ at 660 nm).[739]

L. Germanium, Tin, and Lead

The phenylfluorone method remains the most popular for germanium determination. It was applied to the analysis of lignite ashes,[740] galium slimes,[741] and minerals.[742–744] Some modifications were proposed to increase its sensitivity and selectivity. The reaction of germanium with phenylfluorone was sensitized by the addition of Zephiramine,[745] CTA ($\epsilon = 1.7 \times 10^5$ at 507 nm),[746] and dodecyltrimethylammonium bromide ($\epsilon = 1.72 \times 10^5$ at 503 nm).[747] Some substituted phenylfluorones such as *o*-chlorophenylfluorone ($\epsilon = 1.8 \times 10^5$ at 516 nm),[748] salicylfluorone in the presence of CP ($\epsilon = 1.2 \times 10^5$ at 530 nm),[749] tetrabromosalicylfluorone ($\epsilon = 1.3 \times 10^5$ at 520 nm),[750] and disulfophenylfluorone ($\epsilon = 1.2 \times 10^5$ at 526 nm)[751] were examined.

Trihydroxyphenylfluorones were also discussed as reagents for the determination of tin (ϵ up to 1.6×10^5).[752] Disulfosalicylfluorone in the presence of non-ionic surfactants was reported to give a molar absorptivity of $\epsilon = 1.4–1.6 \times 10^5$.[753]

Ion-associates of anionic complexes with basic dyes play an important role in the spectrophotometric determination of this group of metals. Methods based on the ion-associate of molybdogermanic heteropolyacid with Crystal Violet,[754] Malachite Green,[755] and Methylene Blue[756] were reported to give very high sensitivity ($\epsilon = 4.2–6.2 \times 10^5$) and usually only with interference by silicon. Ion-association of germa-

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nium mandalate,[757] tin 3-nitroalizarinate,[758] or chloride[428] with Malachite Green, Brilliant Green, and Rhodamine 6G, respectively, was described. Analogous methods for lead, which are based on extraction of the ion-pairs of lead iodide or bromide with Fuchsin or Rhodamine B, were discussed.[759,760]

Of the other sensitive reagents for lead, hexaoxacycloazochrom ($\epsilon = 1.5 \times 10^5$ at 720 nm),[761] and Chrompyrazol I ($\epsilon = 1.1 \times 10^5$ at 630 nm)[762] are worth mentioning. The catalytic effect of lead on the oxidation of 4-(3,4-dihydroxyphenylazo)benzenesulfonic acid by persulfate was utilized for a sensitive (5 ng/ml) and selective method of lead determination.[763]

M. Antimony and Bismuth

The methods for the determination of antimony and bismuth mainly involved the extraction of ion-associates of their anionic complexes with basic dyes. Chloride,[764] p-mandelic acid,[765,766] and catechol[767] complexes of antimony were studied. For bismuth, the flotation of its complex with Alizarine Red S associated with Brilliant Green offered very high sensitivity ($\epsilon = 2.2 \times 10^5$).[768]

In a study of eight substituted N-phenyl-2-furylacrylohydroxamic acids as extractants and eight pyridylazo compounds as chromophores, N-p-methoxyphenyl- 2-furylacrylohydroxamic acid and 5-iodo-5-(dimethylamino)-2-(2-pyridylazo)phenol produced the best selectivity and sensitivity ($\epsilon = 6.5 \times 10^4$ at 590 nm) for determining bismuth in environmental samples.[769] 2-Thiobarbituric acid was proposed as the reagent for bismuth ($\epsilon = 2.4 \times 10^4$ at 416 nm).[770] The complexation of bismuth with pyrogallol-based monoazo reagents was studied, and 2,3,4-trihydroxy-4′-sulfoazobenzene was proposed as the reagent of choice ($\epsilon = 5.5 \times 10^4$ at 460 nm).[771] The use of tris-substituted 1,3,5-triazine for the determination of bismuth was also reported.[772]

An interesting method for indirect determination of antimony(III) based on its oxidation by chromium (VI) and subsequent determination of the unreacted chromium (VI) with 1,5-diphenylcarbazide was described.[773,774]

Reaction of bismuth[775-777] and antimony[778] with Bromopyrogallol Red,[775] Pyrocatechol Violet,[776] *o*-hydroxyhydroquinonephthalein,[777] and vanillylfluorone[778] in the presence of non-ionic surfactants was studied, but no significant progress in sensitivity was observed.

N. Boron, Carbon, and Silicone

A rapid development in the methods based on ion-associates of anionic boron complexes was observed. The tetrafluoroborate ion-pair with Methylene Blue was applied to the determination of boron in high purity reagents,[779] copper-alloys,[780] biological materials,[781] and silicon quartz.[782] Similar systems involved mandelic acid and Malachite Green,[783] catechol derivatives and Ethyl Violet,[784] 2,3-dihydroxynaphthalene and Crystal Violet,[785] 2,4-dinitronaphthalene-1,8-diol and Brilliant Green ($\epsilon = 2.2 \times 10^5$ at 637 nm),[786,787] 2,6-dihydroxybenzoic acid with Crystal Violet,[788] Malachite Green,[789] and 4-(4-diethylaminophenylazo)-N-methylpyridinium.[790] Other noteworthy methods involved Azomethine H,[791,792] Azomethine HR,[793] and azomethine derivatives.[794] The most widely used curcumine method was applied after prior extraction with 2-ethylhexane-1,3-diol to the determination of boron in nuclear-grade uranium oxides[795] and steel.[796]

Methods for the determination of cyanide were mostly based on the reaction with pyridine, and the condensation of glutanoic aldehyde formed with barbituric acid ($\epsilon = 1 \times 10^5$ at 580 nm).[797-799] These methods were applied to the determination of cyanide in waste waters. Anthranilic acid instead of barbituric acid was proposed for the determination of cyanide in biological fluids.[800,801] The system based on reaction of cyanide with isonicotinic acid and 3-methyl-1-phenyl-2-pyrazolin-5-one was described for automatic determination of cyanide in waste waters.[802] A few methods for the simultaneous determination of cyanide and thiocyanate, which are based on pyridine-barbituric acid system, were described.[803-805] A novel method for the determination of cyanide based on the formation of a mixed ligand complex with iron (II) and bathophenanthroline was developed.[806] The inhibiting effect of cyanide on the extraction of the lead complex with 5-phenylazo-8-aminoquinoline was

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the basis for its determination in waste and seawaters.[807]

An automatic method for the determination of thiocyanate based on its reaction with chloramine T and coupling with a mixture of 8-picoline and barbituric acid was developed.[808] Indirect methods for the determination of thiocyanate involved the reaction of thiocyanate with mercury (II)[809] or permanganate[810] and the determination of the unreacted reagents. A novel reagent, 5-sulfobutylamino-2′-methoxyphenylanthranilic acid, was proposed for the determination of ferricyanide in galvanic baths.[811]

Methods for the determination of carbon monoxide based on the systems silver-sulfanilic acid,[812] $PdCl_4^{2-}$-cacotheline,[813] and ruthenium (II)-ethylphosphine[814] were described.

Numerous methods for the determination of silicate by the molybdate blue method in natural waters were described.[815–819] Most of these were in flow-injection systems. The same method was applied to the determination of silicon in GaAs[820] and soil extracts.[821] The molybdate blue method was made more sensitive by the formation of flotable[822,823] or stabilized with polyvinyl alcohol ion-pairs with Chrompyrazol II[822] or Malachite Green[823,824] ($\epsilon = 1 \times 10^5$).

O. Nitrogen, Phosphorus, and Arsenic

The application of the commonly used indophenol reaction for the determination of nitrogen was reviewed.[825] An alternative method based on the reaction with hypochlorite, nitroprusside, and salicylic acid was proposed; however, there were no clear advantages over the indophenol method.[826]

Interferences in the various spectrophotometric methods for the determination of nitrite using diazotization-coupling reagents were studied.[827,828] The determination of nitrite was reviewed.[829] Methods for the determination of nitrate, usually in flow-injection mode, involved the reduction of nitrate to nitrite and the determination of the latter.[830–832] Simultaneous determination of nitrate and nitrite in a flow-injection system involves first splitting the sample into two channels. In one channel, nitrate is reduced to nitrite and the sum of nitrate and nitrite is determined using the Griess reaction.[833] In the other channel, only the nitrite is determined using the Griess reaction.

Two methods proposed for the determination of azide ion were based on the reduction of cerium (IV) and the determination of cerium (III) thus formed.[834,835] Some sensitive methods for the determination of hydrazine,[836–838] hydroxylamine,[839,840] and nitric oxides[841–843] were developed.

The molybdate blue method, which is the most widely used method for the determination of phosphorus, was adapted successfully to flow-injection sample processing.[844–848] Two applications based on a LED as the light source were suitable for field monitors.[849,850] The interactions between vanadomolymbdophosphoric acid with basic dye was studied.[851] Some sensitive ($\epsilon = 1–3.5 \times 10^5$) methods, which were based on the ion-pairs of molybdophosphoric or vanadomolybdophosphoric acid, mainly with Malachite Green,[852–857] Rhodamines[855–860] but also with Brilliant Green[861] and Ethyl Violet,[862] were developed.

The spectrophotometric determination of arsenic is based on the same principle as of phosphorus and involved molybdoarsenic blue and and vanadomolybdoarsenic methods.[863–865] Products of reduction of the respective acids could also be associated with basic dyes.[866–868] A flotation-spectrophotometric method based on the ion-pair with Rhodamine B offered very high sensitivity, $\epsilon = 8.8 \times 10^5$.[869] It must be emphasized that, in all these methods, phosphorous and silicon cause interferences and tedious preliminary separation is usually required. Better selectivity is offered by ion-associates of arsenic complexes with thiol derivatives with basic dyes.[870,871] A novel, extremely sensitive ($\epsilon = 9.4 \times 10^5$) amplification method was developed.[872] Selective photometric determination of arsenic in high purity chemicals[873] was described.

P. Sulfur, Selenium, and Tellurium

A few methods for the determination of sulfate, which were based on the decomposition of the barium complex with dimethylsulfonazo[874,875] or Orthanyl K,[876] were adapted to FIA. An in-

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teresting indirect method, which is based on the substitution of chromate in barium chromate by sulfate and subsequent determination of chromium with diphenylcarbazide, was developed.[877]

A method for the determination of sulfide employing its ability to reduce molybdophosphoric acid to molybdate blue was described.[878] Another sensitive method involved the ligand-exchange reaction in the silver complex with Cadion 2B.[879] Spectrophotometric determination of polythionates and thiosulfate was recently reviewed.[880]

The determination of selenium at trace levels by various analytical techniques was reviewed.[881] Novel reagents include 4,5,6-triaminopyrimidine,[882] dithioxamide,[883] and 2-aminodiphenylaniline.[884] Two sensitive indirect methods were based on the reduction of ferrocyanide[885] or ferrocene[886] by selenium (IV) and the determination of iron (II) formed by the phenanthroline method.

The extraction-spectrophotometric determination of tellurium with dithiocarbamic acid derivatives with molten naphthalene[887,888] was developed. New reagents reported for the determination of tellurium involved *iso*-butyldithioantipyrilmethane[889] ($\epsilon = 5.1 \times 10^4$ at 358 nm) and tetramethylthiourea.[890] A sensitive flotation-spectrophotometric method ($\epsilon = 1.4 \times 10^5$ at 640 nm) based on the flotation of tellurium iodide complex with Nile Blue A was described.[891]

Q. Halogens, Oxygen

Methods for the determination of fluoride are usually indirect and were based on the decomposition of intensely colored metal complexes in the presence of fluoride. The systems La-Alizarine Complexon,[892-894] Zr-Eriochromcyanine R,[895,896] Zr-Alizarine S,[897] Th-Chromazurol S[898] were the most widely used. Two direct methods for the determination of fluoride were based on the extraction of ternary complex Al-F-Xylenol Orange[899] and Zr-F-Xylenol Orange.[900]

Chloride was mostly determined indirectly by the determination of thiocyanate ions, which are released from the mercury thiocyanate complex when substituted by chloride.[901-904] The same principle was employed in the flow-injection determination of chloride in atmospheric aerosols, but a mercury-chloranilic acid complex was used.[905] Some of the reagents that were applied to the determination of chlorine included 4-nitroaniline,[906] 2,7-fluorenediamine,[907] and Michler's thioketone.[908] Chlorine dioxide was determined in potable water with Chlorophenol Red.[909] Dimedone bisthiosemicarbazone[910] and cyclopentane-1,3-dione bis(4-methylthiosemicarbazone)[911] were used for the rapid determination of chlorate. Perchlorate was determined after extraction of ion-pairs formed with amiloride,[912] batoferroine,[913] Malachite Green,[914] and Brilliant Green.[915,916]

Bromide was determined in waters in FIA mode by the well-established Phenol Red method.[917] New methods proposed for the determination of bromate were based on the oxidation of Pyrogallol Red,[918] 2-oximinodimedone dithiosemicarbazone,[919] and 1,3,4-trihydroxyanthraquinone-2-carboxylic acid.[920]

As far as the determination of iodide is concerned, no competetive method for the well-established iodine-starch method was reported. The only noteworthy approach was the flow-injection determination of iodide.[921] For spectrophotometric determination of periodate guanylhydrazones of salicyl[922] and 3,4-dihydroxybenzaldehyde[923] and dithiosemicarbazones of 2-oximinodimedone[919] and cyclohexane-1,3-dione,[924] phthalimide and 1,3-indandione[925] were proposed. A method for the determination of iodine in iodated salt using diphenylcarbazide was developed.[926]

Indigo di-[927] and trisulfonic acids[928] were proposed as reagents for the spectrophotometric determination of ozone. Other reagents proposed for the same purpose involved bis(terpyridine)-iron (II) complex[929] and 1,1,-diphenylethylene.[930]

A simple indirect method for the determination of hydrogen peroxide, which is based on the reduction of copper (II) by hydrogen peroxide and subsequent determination of copper (I) with neocuproine, was developed.[931] Oxidation of Bindschedler's Green leucobase[932] and the catalytic effect of hydrogen peroxide on antipyrine[933] were utilized for the flow-injection spectrophotometric determination of hydrogen peroxide. Extraction of hydrogen peroxide with ethyl acetate was proposed to circumvent problems arising

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when determining hydrogen peroxide in intensely colored solutions.[934] A comparison study on five procedures on the determination of hydrogen peroxide in aqueous solutions was published.[935]

V. ADVANCES IN SPECTROPHOTOMETRIC METHODS FOR ORGANIC CONSTITUENTS

A shift away from inorganic analysis toward the photometric determination of organic constituents, especially those of interest in clinical and pharmaceutical chemistry, has been observed during the past decade. It was prompted by the development of powerful signal-processing techniques like derivative spectrophotometry and multiwavelength analysis, which facilitated the resolution of complex mixtures with highly overlapped spectra. However, due to high selectivity, these techniques allow for direct determination of the compound of interest in the solutions, sometimes even permitting multicomponent analysis. The usefulness of these techniques has so far been demonstrated mainly for synthetic samples where all the potential interferences of the compounds are known. In real sample analysis, these methods are prone to error and need verification by an independent technique, usually HPLC with mandatory confirmation of peak purity. To date, this is not done very often, although a distinct trend has been observed.

Methods based on reaction of the analyte with a color-producing reagent are more selective, but even then a specific method for one component is rare. Usually, a group of compounds that have the same functional group is involved. This category of methods often becomes the standard for the analysis of pharmaceuticals and is recommended by various Pharmacopeias.

Table 10 shows a selection of methods that involve the use of color-forming reagents and were applied to the analysis of real samples. It must be emphasized, however, that proof of the accuracy of these methods is hardly ever provided.

VI. CONCLUSION

Despite being regarded by many analysts as obsolete, spectrophotometry still enjoys great popularity, which, in some fields, even seems to be growing. Although the reference list appears lengthy, it only covers a small percentage of the papers published in the last decade. Many of these papers, however, offer methods with no real advantages over most existing methods. In many reports in the analytical journals, the development of a new method seems to be a goal in itself. Interference studies usually reveal iron as the interferent, which precludes the application of the method to trace analysis. The large number of reports of dubious usefulness makes it hard to find the developments with high potential.

It seems that further advances in the synthesis of new organic reagents will occur, but whether they will be able to bring about a breakthrough in analytical spectrophotometry is rather doubtful. To ensure the relevance of a method based on a newly synthesized reagent, its application to real sample analysis must be demonstrated. The accuracy of an analytical method should be confirmed by an independent method or by the analysis of certified reference materials. Detailed comparative studies of the method developed not only with the well-established photometric methods, but also with atomic-spectrophotometric methods are uncommon in the analytical literature and are strongly encouraged. The incorporation of novel reagents into routine analytical practice, which is now hampered by the lack of convincing evidence of the superiority of a new reagent or method over existing ones, would enhance interest.

The gradual replacement of spectrophotometry by AAS and ICP AES in inorganic analysis continues in modern laboratories. However, growing interest in automatic sample processing can place spectrophotometry as a convenient detection technique due to the relatively low cost of the equipment and, above all, its far easier and cheaper maintenance. The drawback of poor selectivity is more often overcome by powerful signal-processing techniques. These all make spectrophotometry a competitive technique, if not in ultratrace, at least in trace analysis.

Instrumental developments have opened new areas of application that need to be filled with the development of new spectrophotometric methods. The most promising field appears to be spectrophotometric PDA detection in HPLC cou-

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TABLE 10
Selected Applications of Spectrophotometry in the Determination of Organic Compounds

Analyte	Color reagent	λ, nm	ε × 10^{-4}	Range, mg/l	Sample	Ref.
Albumin	Chromazurol S/Al(III)	630		1.3–25	Serum	936
Amidopyrine	Tetracyanoethylene	420	0.19	10–60	Drugs	937
Amiloride	3-Methyl-2-benzothiazolinone hydrazone/Ce(IV)	545	3.78	12.5–150 μg	Drugs	938
Amines	p-Chloranil	491–563			Organic compounds	939
	2-Iodylbenzoate	525		4–32	Sulfadrugs	940
	Asorbic acid, AC_2O	273	3.95–5.35		Drugs	941
p-Aminophenol	3-Cyano-N-methoxypyridinium perchlorate/acetate	410	2.95	0.4–2.4 g/l	Paracetamol tablets	942
	3-Cyano-N-methoxypyridinium perchlorate/chloramine T	448	1.7	1.6–8.0 g/l	Paracetamol tablets	942
Aminoquinolines	Co(II)/SCN^-	625			Drugs	943
Ampicillin	Pd(II)/o-hydroxyhydroquinonephthalein	630			Dry syrup	944
Antimalarials	Chloranilic acid	522		40–200	Tablets	945
Antipyrine	Tetracyanoethylene	440	0.155	30–80	Drugs	937
Apramycin	Bromothymol Blue	430		1–5 m*M*	Drugs	946
Atropine	Chloranilic acid	535		25–200	Drugs	947
Benzothiadiazine diuretics	7,7,8,8,-Tetracyanoquinonedimethane, sodium acetate	578		0.7–6.0	Drugs	948
Benzothiadiazines	OH^-/ethyl acetoacetate	425–430	1.26–3.46	2–48	Drugs	949
Bumetanide	3-Methyl-2-benzothiazolinone hydrazone/Fe(III)	660	2.49	10–100 μg	Drugs	938
Carvone	Oximation/iodine	300		10–50	Drugs	950
	Oximation/chloranil	430		10–50	Drugs	950
Cephalxin	Pd(II)/o-hydroxyhydroquinonephthalein	630			Dry syrup	944
Cephalosporins	Molybdophosphoric acid		2.48–9.05		Drugs	951
	Ammonium molybdate	670		25–30	Drugs	952
	Ninhydrin/H_2SO_4	458		2.5–30	Injections	953
	OH^-/N,N-dimethyl-p-phenylenediamine/Fe(III)				Penicillins	954
Chloramphenicol	OH^-/sodium cobaltinitrite/H_3PO_4	240		10–30	Drugs	955
Clavulanic acid		312		d.l. 0.2	Imidazole	956
Codeine	2,3-Dichloro-5,6-dicyano-p-benzoquinone	460	0.25	20–90	Drugs	957
Corticosteroids	Oxime formation/iodine	300		10–50	Tablets	958
	Oxime formation/chloranil	435		20–80	Tablets	958
γ-Cyclodextrin	Bromocresol Green	630		Up to 700	Enzymatic products from starch	959
Diethyldithio-phosphate	$Pb(ClO_4)_2$	293		Up to 40	Flotation liquors	960
1,2-Diphenols	Phenylfluorone/Fe(III)	630	17.0		Drugs	961
Emetine	2,3-Dichloro-5,6-dicyano-p-benzoquinone	460	1.54	6–22	Drugs	957
Epicillin	Direct after acidic hydrolysis				Plasma	962
Ethynyl steroids	$HgCl_2$/dithizone	480			Drugs	963
Flufenamic acid	$K_3Fe(CN)_6$/NaOH	464	0.29		Capsules	964
Frusemide	3-Methyl-2-benzothiazolinone hydrazone/Fe(III)	630	1.76	25–250 μg	Drugs	938
Glucose	Enzymatic degradation to H_2O_2, Ti(IV)/2-[(5-bromopyridyl)azo]-5-(N-propyl-N-sulfopropylamino)phenol	539		3–279 mg/dl	Serum	965
Griseofulvin	Oximation/iodine	300		10–50	Drugs	950
	Oximation/chloranil	430		10–50	Drugs	950
Hemoglobin	Phenothiazine			4–500	Plasma	966
Hydralazine	Tetracyanoethylene	410	0.99	2–40	Drugs	937
Hydroperoxides	Iodide/cadmium acetate	358	2.97		Biological	967
8-Hydroxyquinolines (halogenated)	4-Aminoantipyrine, hexacyanoferrate(III)	500	1.20–1.29	2–32	Tablets, syrups	968
Imidazolines	2,6-Dichlorophenolindophenol	588–603		0.001–0.010	Tablets, nasal drops	969
Isoniazid	Metol/vanadate/Fe(III)				Drugs	970

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TABLE 10 (continued)
Selected Applications of Spectrophotometry in the Determination of Organic Compounds

Analyte	Color reagent	λ, nm	ε × 10^{-4}	Range, mg/l	Sample	Ref.
Isonicotinic acid hydrazide	Tetracyanoethylene	380	0.6	1–10	Drugs	937
Kanamycin	Bromothymol Blue	460		1–5 m*M*	Drugs	946
Ketosteroids		550		5–30	Drugs	971
	Oximation/iodine	300		10–50	Drugs	950
	Oximation/chloranil	430		10–50	Drugs	950
Mefenamic acid	$K_3Fe(CN)_6$/NaOH	464	0.19		Capsules	964
Menthone	Oximation/iodine	300		10–50	Drugs	950
	Oximation/chloranil	430		10–50	Drugs	950
Meperidin	Bromocresol Green	425		2–20	Drugs	972
Methamphetamine	Tetrabromophenolphthalein ethyl ester	570			Urine	973
Methylpentynol	Mercuric acetate	277–280	3.79	4–40	Tablets, drops	974
Minocycline	$ZrCl_4$/NaF/SDS/ o-hydroxyhydroquinonephthalein	515		0.5–4.0	Drugs	975
Penicillins	Ammonium molybdate	670		35–90	Drugs	976
Peroxides	Iodide	360			Polysorbate 60	977
Phenazopyridine	2,6-Dichloroquinone chlorimide, 2,6-dibromoquinone chlorimide, acetate	700		2–25	Tablets	978
Phenelzine sulfate	Tetracyanoethylene	395	0.26	20–80	Drugs	937
Phenols	Iodine monobromide			0.008–0.16 d.l. 0.001	Natural waters Waste waters	979
	m-Phenylene diamine/metapriodate	480–520		1–12	Tablets, syrups	980
Phenothiazines	Morpholine/N-bromosuccinimide	660			Drugs	981
	Nitroso-R-salt	381–395			Drugs	982
	Morpholine/KI-I_2	662–690	1.1–7.2		Drugs	983
	Cobaltinitrite/H_3PO_4	500–530		8–40	Drugs	955
	Molybdophosphoric acid	510–540		100–2000 μg	Drugs	984
β-Phenylpurivic	Al(III)/9-(2'-carboxyphenyl)-4,5-dibromo-2,3,7-trihydroxy fluorone	570	8.4		Urine	985
Phospholipids	Enzymatic degradation to H_2O_2/4-aminoantipyrine	510			Amniotic fluids	986
Pilocarpine	2,3-Dichloro-5,6-dicyano-p-benzoquinone	460	0.22	20–80	Drugs	957
	Chloranilic acid	527.5		10–80	Drugs	947
Saccharin	Azure B/NaH_2PO_4/citric acid	0.24			Soft drinks	987
Sialic acid	Acidic hydrolysis/Ehrlich's reagent	525			Serum	988
Strychnine	Chloranilic acid	535		30–240	Drugs	947
Sulfa drugs	3-Methylbenzothiazolin-2-one hydrazone	565–620	0.2–0.6	5–100	Drugs	989
Surfactants	Tetrabromophenolphthalein ethyl ester K salt	620			Water	990
Tetracyclines	$CuCl_2$/OH^-	395–410		Up to 20	Drugs	991
	Cobaltinitrite/H_3PO_4	243–294		10–30	Drugs	955
Thiazide diuretics	OH^-/periodate/ 3-Methyl-2-benzothiazolinone hydrazone	550	1.13–6.07		Drugs	938
Thiols	Formation and hydrolysis of S-nitrosothiols	544	4.11	1–35 μ*M*	Protein	992
Thioxanthenes	Iodine	275, 360		1–20	Drugs	993
Thiram residues	$CuClO_4$4MeCN/MeCN	420		Up to 8	Grain	946
Tranquilizers	2,3-Dichloro-5,6-dicyano-p-benzoquinone	460			Tablets	994
	Chloranil	550			Drugs	995

pled with efficient data-processing systems. This method offers unlimited potential to the determination of organic compounds even in complex samples.

Future trends involve further improvements in spectrophotometric measurement detection limits, which are closely related to advances in laser technology. The potential here, as indicated by preliminary reports, is very great and may bring spectrophotometric analysis to ultratrace levels.

ACKNOWLEDGMENT

The authors thank Mrs. Joanna Szpunar-Lobińska for her help with collecting the references and the word-processing of the manuscript.

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