Advances in miniaturized UV-Vis spectrometric systems

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UV-Vis spectrometry has become a widespread analytical technique due to its inherent features (e.g., simplicity, ease of operation, convenience and economy). The necessity to decrease the consumption of valuable and scarce samples, together with the application of novel sample-retention technologies, the improvement of materials and the development of microfluidics, has enabled development of a new generation of miniaturized UV-Vis spectrometric systems and accessories. This article surveys the state of the art on microsample-quantitation systems in UV-Vis spectrometry.

Keywords: Drop-based system; Liquid-core waveguide; Microcell system; Microfluidics; Microsample quantitation; Micro-total analysis system; Miniaturization; Sample retention; UV-Vis spectrometry; Variable path-length system

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1. Introduction

UV-Vis spectrometry is widely employed in different scientific areas all around the world, especially in biochemistry and analytical chemistry, due to its simplicity, flexibility, low cost and convenience. Hundreds of analytical methods concerning UV-Vis spectrometry can be found in the literature, including official and standard methods of analysis. However, the current necessity of reducing the amounts of sample and/or reagents needed to perform an analytical measurement, especially when scarce samples or toxic organic solvents are involved, has promoted the development of microvolume UV-Vis spectrometric instrumentation.

Miniaturization of detection, separation and sample preparation constitutes a clear trend in analytical chemistry. Integration of different analytical steps, reliability, portability and ease of operation can be considered the main factors towards the development of miniaturized analytical devices [1,2]. Also, miniaturization in analytical chemistry is commonly accompanied by low consumption of sample and reagents, and so negligible generation of wastes. Miniaturized analytical systems that fulfill the latter can be ascribed to green analytical chemistry [3]. Advances in microvolume UV-Vis spectrometry mainly derive from efforts to miniaturize the sample compartment, as described below. Nevertheless, it should not be forgotten that these developments in microvolume quantitation have also been accompanied by improvements in radiation sources and detectors, and the extended use of fiber optics.

In the literature, there are a few articles dealing with the features of specific microvolume UV-Vis spectrometric systems [4,5] or concerning strategies for the improvement of sensitivity in UV-Vis spectrometry [6]. However, none of them focuses on miniaturization of UV-Vis spectrometric systems or provides an updated description of the different possibilities in this field.

In the present work, we provide an overview of the analytical tools developed for microsample UV-Vis spectrometric quantitation. We emphasize miniaturization of analytical instrumentation and employment of novel materials and systems that allow replacement of standard cuvettes with the general purpose of providing analytical characteristics with μL or nL sample consumption comparable to conventional analytical systems.

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2. From conventional to microvolume UV-Vis spectrometry

As mentioned above, conventional UV-Vis spectrometry has been greatly employed for the analysis of a large variety of samples, with several official and standard methods of analysis involving determination of target analytes in samples including waters, foods, beverages and pharmaceuticals. For such UV-Vis spectrometric analysis, 10-mm optical path cuvettes are typically employed as absorption cells, although cuvettes showing larger (or shorter) optical path can be useful under certain circumstances. However, these cuvettes suffer from practical limitations, which may be significant for accurate UV-Vis spectrometric analysis of scarce samples. Thus, conventional cuvettes yield a relatively poor path length (10 mm) for the large sample volume needed to perform UV-Vis spectrometric analysis (2–3 mL), bearing in mind that sensitivity is directly proportional to the path length of the absorption cell in accordance with the Lambert-Beer law. ''Semi-micro'' cuvettes have also been employed for the analysis of limited sample volumes, keeping a 10-mm path length but reducing the sample-volume requirements to 0.65 mL. The increase in the ratio of optical path length to sample volume has been a way to achieve acceptable sensitivity with reduced sample consumption. In this sense, U-cells [7], Z-cells [8], and bubble-shaped cells [9] have been developed for UV-Vis spectrometric systems coupled with separation techniques [e.g., liquid chromatography (LC) or capillary electrophoresis (CE)]. Also, capillary tubes with wall transmission were proposed for near-infrared (NIR) spectroscopy measurements [10].

Further advances have been made towards improvement and miniaturization of UV-Vis spectrometry by making use of new materials and technologies that allow the employment of microvolumes or nanovolumes of samples and/or organic solvents with sensitivity close to that provided by conventional UV-Vis spectrometers, as described below.

3. Microsample quantitation

One of the challenges in the development of miniaturized analytical devices is the miniaturization of detectors [1]. As far as UV-Vis spectrometry is concerned, the decrease in the sample volume needed to carry out a single measurement is the key to extending even more the applicability of this widespread analytical technique. In this sense, different UV-Vis spectrometric systems and accessories have been developed in recent years, and some of them can currently be found in the market, including confined drop-based systems, liquid-core waveguides (LCWs), microcells, UV-transmissive pipette tips and variable path-length systems. We describe each of these commercial alternatives to conventional UV-Vis spectrometric systems briefly below and summarize their characteristics of detection volume and path length in Table 1. Also, we describe other microvolume systems [e.g., drops and films that act as windowless optical cells, polymeric porous tubes, microfluidic systems and cavity ring-down spectrometers (CRDSs)].

3.1. Confined drop-based systems

Two different designs of confined drop-based UV-Vis spectrometers have been sample-retention systems based on surface tension and hydrophobicity, respectively.

The use of the surface tension of a low sample volume to hold it in place between two planar surfaces was proposed and patented by Robertson in 2003 [11]. This sample-retention technology is employed in microvolume UV-Vis spectrometers marketed by Thermo Fisher Scientific (Nanodrop ND-1000, Nanodrop 2000, Nanodrop 2000C and Nanodrop 8000) [12]. These microvolume UV-Vis spectrometers allow the spectrometric measurement of sample volumes of 0.5–2 μL in a few seconds. In these systems, the μL sample volume is confined by surface-tension forces in the optical path of the optical fibers embedded in stainless-steel surfaces drawing up the sample-measurement column. The path length is mechanically controlled between the ends of two optical surfaces. This confined drop-based system is depicted in Fig. 1A. Light from a xenon flash lamp passes through the sample by means of the above-mentioned optical fiber and the intensity of the transmitted light is measured by means of a CCD detector.

Shimadzu has introduced a UV-Vis spectrometer, named BioSpec nano, based on the same sample-retention system [13]. Furthermore, measurement and cleaning are conducted automatically with this instrument. However, the path length of this system depends on the sample volume, the optical path length of 0.7 mm being larger, as shown in Table 1.

A different system marketed by GE Healthcare as Nanovue uses a hydrophobic sample-plate coating to hold a sample microdrop in place during the measurement cycle [14]. The sample is pipetted onto the hydrophobic surface and flattened by lowering the sampling head. A short path length (0.2 mm or 0.5 mm) is then automatically adjusted.

Both confined drop-based systems allow the analysis of microvolume samples in an easy way without the need to use cuvettes or capillaries. Moreover, they offer quick and easy cleaning, so minimizing the risk of cross-contamination. However, physical properties of the microvolume sample (e.g., boiling point and vapor pressure) should be taken into account when using these UV-Vis spectrometric systems, since the microdrop is partly exposed to air during drop deposition and UV-Vis spectrometric measurement, so evaporation could occur.

Table 1. Characteristics of commercial microvolume systems used for UV-Vis spectrophotometric measurements

Microvolume systems		Detection volume	Path length
Confined drop			
NanoDrop	Thermo Scientific	0.5, 1 and 2 μL	0.05, 0.2 and 1 mm
BioSpec Nano	Shimadzu	1 and 2 μL	0.2 and 0.7 mm
NanoVue Plus	GE Healthcare	0.5–5 μL	0.2 and 0.5 mm
Liquid-core waveguide			
LWCC	World Precision Instruments	5, 12.5 and 25 μL	20, 50 and 100 mm
MicroLWCC		2.4 and 12	10 and 50 mm
SpectroPipetter		2 μL	10 mm
DipTip		⩾ 20 μL	2, 5 and 10 mm
Microcell			
NanoCell	Thermo Scientific	0.7–5 μL	0.2 and 1 mm
TrayCell	Hellma	0.7–5 μL	0.2 and 1 mm
LabelGuard Microliter Cell	Implen	0.7–10 μL	0.2, 1 and 2 mm
In-tip measurement			
Picodrop	Genetic Research Instrumentation	2 μL	1 mm
Variable path length			
SoloVPE	C Technologies Inc.	⩾ 3 μL	0.01–20 mm

3.2. Liquid-core waveguides

Light can be efficiently propagated through a capillary filled with a fluid when the refractive index of the capillary is less than that of the fluid inside it. Under these conditions, the radiation propagates through the fluid core by total internal reflection (TIR) [15]. This phenomenon was exploited by Gambling et al. to patent a liquid-core fiber-optic waveguide valid for organic solvents [16]. Furthermore, the development of materials showing lower refractive index than water {e.g., the family of fluoropolymers Teflon AF (2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole) introduced by DuPont [17]} allowed the development of LCWs also valid for aqueous samples [18]. LCWs provide enhanced sensitivity since radiation is efficiently detected and the background noise is minimized.

The use of LCW-based long path-length systems was mainly exploited in the ultratrace determination of environmental pollutants [15,19]. Commercial systems marketed by World Precision Instruments (WPI) allow the employment of large optical path lengths (20–5000 mm) with sample volumes up to 1250 μL [20]. Nevertheless, WPI has marketed different low-volume LCWs that can be more interesting for microvolume-sample quantitation, showing path lengths in the range 20–100 mm with a detection volume less than 25 μL. In addition, a low-volume flow cell, named microLWCC, allows UV-Vis spectrometric measurements with a path length of 10 mm or 50 mm using 2.4 μL or 12 μL of sample solution, respectively.

A microcell within a fiber-optic plunger system that shows 10-mm path length and requires only 2 μL to perform a measurement is also commercially available under the name SpectroPipetter. Patented by Liu [21] and marketed by WPI, the SpectroPipetter system is shaped like a micropipette, although its configuration is not as simple as it appears. The UV-Vis spectrometric sampling device comprises a sample cell placed inside the tip of the SpectroPipetter, fiber optics that carry light to and from the sample drop and acts as a plunger, and a pusher arm that draws the microvolume sample (2 μL) into the microcell and expels it once the UV-Vis spectrometric measurement is completed. The SpectroPipetter is depicted in Fig. 1B. The SpectroPipetter is used in combination with an external light source and an analyzer through a coupler and optical fiber. Light is transmitted axially through the sample in this system, thus increasing path length. The microvolume sample fills an inflexible tubular vessel fabricated from Teflon AF. A mirror placed at the end of the tip reflects the light beam, so doubling the light path through the sample. The SpectroPipetter system provides a ratio of path length to detection volume three orders of magnitude greater than conventional cuvettes, thereby constituting a powerful system for UV-Vis spectrometric determination of target analytes in scarce samples at low concentrations. However, this system is quite delicate due to the nature of the optical components.

Another system related to the Spectropipetter is the DipTip, a miniaturized transmission probe also marketed by WPI. Like the Spectropipetter, DipTip has two optical-fiber cables coupled to the radiation source and the detector. This system also uses a mirror to return the light. UV-Vis spectrometric measurements can be performed by immersing the tip in 20 μL of sample solution with path lengths in the range 2–10 mm.

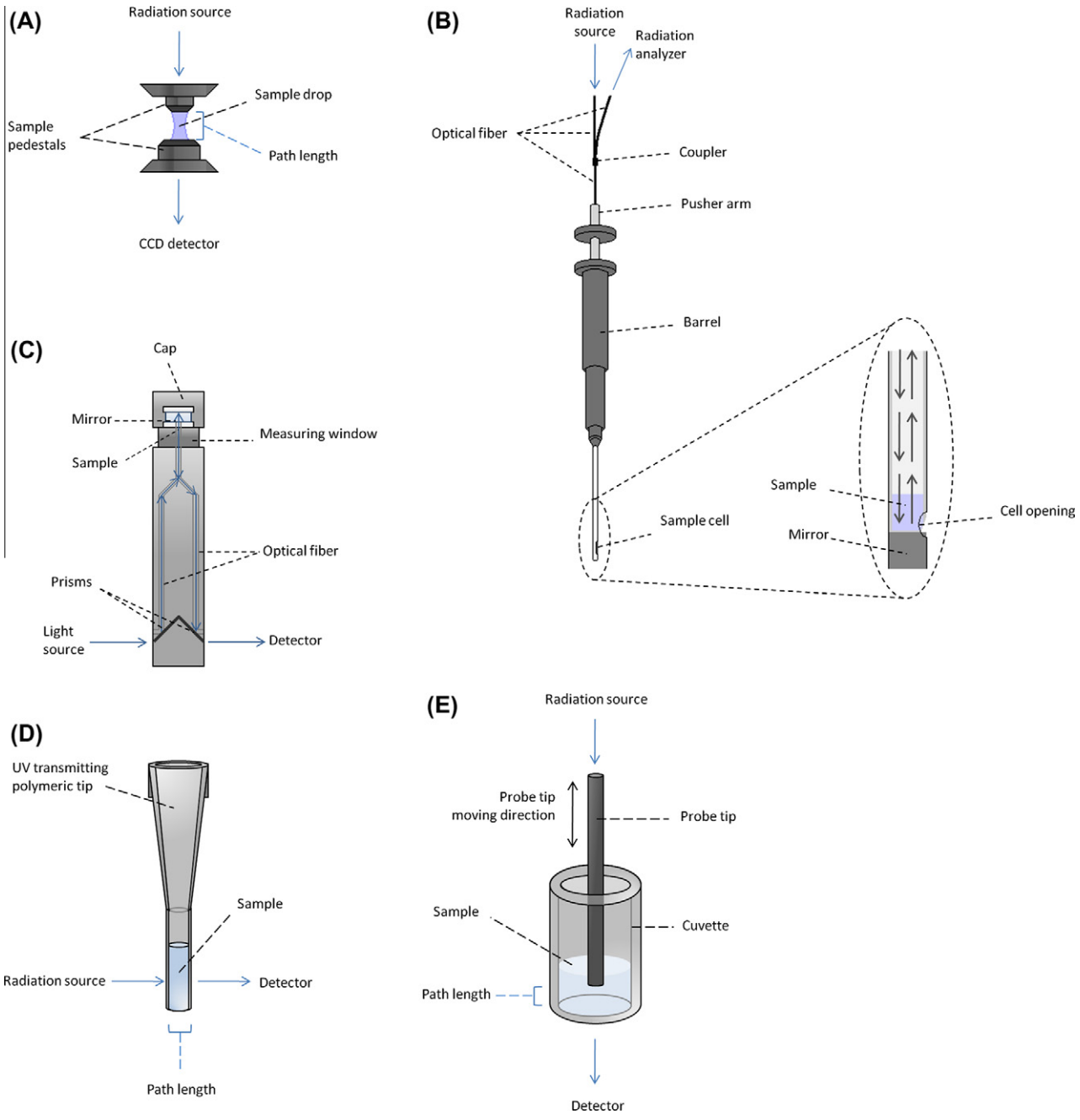

Figure 1. Commercial microvolume systems. (A) Confined drop-based UV-Vis spectrometer with surface-tension retention system; (B) Spectro-Pipetter system based on liquid-core-waveguide (LCW) technology; (C) microcell system; (D) in-tip measurement system; and, (E) variable path-length system.

3.3. Microcell systems

Microcells can also be used as UV-Vis spectrometric accessories. Even though these systems have been developed for life-science purposes, analytical chemists can also exploit their features. So far, a few microcell systems {i.e. TrayCell (Hellma) [22], Labelguard (Implen GmbH) [23] and nanoCell (Thermo Scientific) [24]} are commercialized. Fig. 1C depicts a microcell system. In these systems, a sample volume of 0.7–10 μL is pipetted directly onto the center of the active measurement window of the cuvette and sandwiched between an optical fiber and a mirror (placed on a cap). The use of different lids allows formation of different liquid columns of defined path lengths in the range 0.2–2.0 mm. The appropriate volume of sample (which depends on the path length required) is deposited onto the measuring window and capped. The cap compresses the sample drop, thus defining the optical path length. The light beam is deflected inside the microcell,

thanks to the internal prism, and conducted to the sample microvolume through optical fiber. The transmitted light beam is reflected by the mirror and conducted towards the detector via the optical fiber and a second internal prism.

Microcells show a size equivalent to conventional cuvettes, so that they can be placed in the sample compartment of conventional UV-Vis spectrometers. In addition, evaporation of the microvolume of sample/organic solvent is reduced, since it is not exposed to air during the UV-Vis spectrometric measurement.

3.4. In-tip measurement system

The development of polymer technology makes possible the use of UV-light-transmitting polymers to replace conventional quartz cuvettes used typically in UV-Vis spectrometry. Polymers having good UV and visible-light transmission are achievable by polymerization of at least one compound having a substantially non-UV absorbing core group (i.e. linear or branched aliphatic hydrocarbons that may contain an aliphatic ring or polydialkylsiloxanes) [25].

A copolymer based on cyclic olefins showing high transparency in the UV range is commercially available as TOPAS 8007X10 [26]. The high transparency of this material was exploited by Redfern, who patented UV-transmissive pipette tips as containment vessels for microvolume samples [27,28].

In these inventions, the narrower section of the tip (Fig. 1D) shows constant diameter inside and out. A column of liquid is formed when sufficient sample volume fills this section of the pipette tip. The use of two optical fibers (one fiber being the source and the other the receiver) mounted coaxially and perpendicular to the tip allows UV-Vis spectrometric analysis of the sample solution.

Polymeric materials that can transmit light between 220 nm and 850 nm have been employed by PicoDrop [29] to make disposable pipette tips (UVpette tips). A 2-μL sample is aspirated with an UV-transmitting polymeric tip, and the pipette tip is then placed between two optical fibers for a single UV-Vis spectrometric measurement. The path length (1 mm) is defined by the internal diameter of the tip.

The use of disposable tips eliminates the need to transfer the sample to another container in order to perform a UV-Vis spectrometric measurement, and also avoids any possible cross-contamination and evaporation of the sample microdrop, as described above with drop-based systems. However, the need to replace the tip for subsequent analysis may increase uncertainty about the path length, which may give rise to measurement errors. Furthermore, additional cost derives from the need to renew the consumable UVpette tips.

3.5. Variable path-length system

The systems described above allow the absorbance measurement of sample solutions to be made at fixed path lengths. Another possibility easily derived from the Lambert-Beer law is employment of variable path lengths to perform UV-Vis spectrometric measurements. This concept was recently patented by Salerno et al. [30].

The development of a probe for use with a UV-Vis spectrometer and a light source in making UV-Vis spectrometric measurements at variable path lengths opens up new possibilities for UV-Vis spectrometric analysis. A novel commercial system that takes advantage of the use of tunable, controlled path length was marketed by C Technologies Inc. as SoloVPE (variable path-length extension) [31]. Unlike conventional UV-Vis spectrometers, which use fixed path-length cuvettes, the SoloVPE provides fine control over the path length used in measurements across a wide range of path lengths (0.01–20 mm) with a path-length resolution of 0.005 mm. Fig. 1E shows this system.

The light beam passes through an optical fiber (called a Fibrette) and the sample solution. The optical fiber can be mechanically moved up or down relative to the bottom of the sample vessel holding the solution. The motion of the Fibrette is precisely and accurately controlled by computer. The path length is defined by the position of the probe tip with respect to the bottom of the sample vessel. The light transmitted through the sample solution is finally captured by a detector mounted below the sample vessel.

The use of such a large path-length range allows this system to perform measurements over a wide range of concentrations without the need to dilute concentrated samples. It should be noted that the lowest sample volume needed to perform a measurement is 3 μL. However, the available path lengths that can be used with this volume are not specified and are presumably lower than those used in the commercial systems described above. Nevertheless, at 20 mm, the SoloVPE has the largest path length available, although the sample volume needed to carry out measurements under these conditions is not specified.

3.6. Drops, liquid films and porous tubes as collectors and optical cells

The use of μL-volume drops and liquid films as both extractant phases and windowless sample compartments was introduced by Dasgupta's group in 1995 [32–34]. Radiation from a light-emitting diode (LED) passes through the liquid drop [35] or film [36] and the transmitted radiation is conducted by means of an optical fiber to a detector photodiode to obtain the corresponding absorbance measurement. These systems allow extraction and preconcentration of target analytes with *in-situ* derivatization, when necessary, and, more importantly, simplification and integration of the different steps needed to perform a chemical analysis. The windowless nature of these systems can solve problems

associated with conventional cuvettes (e.g., scattering of radiation). However, sensitivity is limited by path length, which is defined by the diameter of the drop or the thickness of the liquid film. Fig. 2A shows a collection system where a drop acts as sampling interface for soluble volatile compounds.

An analogous system was also employed to perform miniaturized liquid-liquid extractions, so a microvolume of organic solvent could be used to carry out the extraction of analytes of interest by direct contact with the sample solution containing absorbing analytes or derivatives during an appropriate extraction time [37]. This system provides the possibility of performing several discrete steps with automated operation:

(1) removal of the previous drop;
(2) cleaning the tip;
(3) controlled generation of a microdrop of organic solvent;
(4) extraction;
(5) washing of the aqueous solution; and,
(6) monitoring the absorbance of the enriched organic phase.

The analytical signal is obtained by placing an LED on one side of the drop and a receiver optical fiber on the opposite side to lead the radiation beam to a photodiode-based detector. The great potential of such drop-based systems and the applications derived from their use were covered with detail in two excellent review articles [33,34].

Integration of a flow cell within an LED source was proposed [38]. Employment of an automatic injector and a peristaltic pump with this system allows the sample solution to flow through the tube. Microdrops containing absorbing analytes or derivatives are continuously produced at the tip of the tube with high reproducibility, in such a way that the path length of the system may be controlled. An approximate path length of 2 mm was reported. An LED-based flow-through system is depicted in Fig. 2B.

Furthermore, cylindrical liquid-filled porous tubes made of Teflon AF [39], polypropylene and polytetrafluoroethylene [40,41] were proposed for the collection and the preconcentration of volatiles. Light is transmitted axially through the liquid contained in such porous cylinders by an LED source and received by a photodiode detector through an optical fiber. As explained above, Teflon AF has a low refractive index that allows use of large optical path lengths (up to 30 cm). However, this amorphous material suffers from reduced permeation of volatile analytes, compared with polypropylene and polytetrafluoroethylene, which do not function as LCWs but they can be useful when relatively short lengths (1–5 cm) are used.

The application of acoustically-levitated droplets in combination with UV-Vis spectrometry as detection system is also remarkable. Among the benefits of using ultrasonic levitation is its ability to perform contactless measurements and to integrate different steps of analytical processes. Acoustic levitators are commonly employed to achieve stable levitation of microdrops in a node of a standing wave produced between transducer and curved reflector. The applicability of ultrasonic

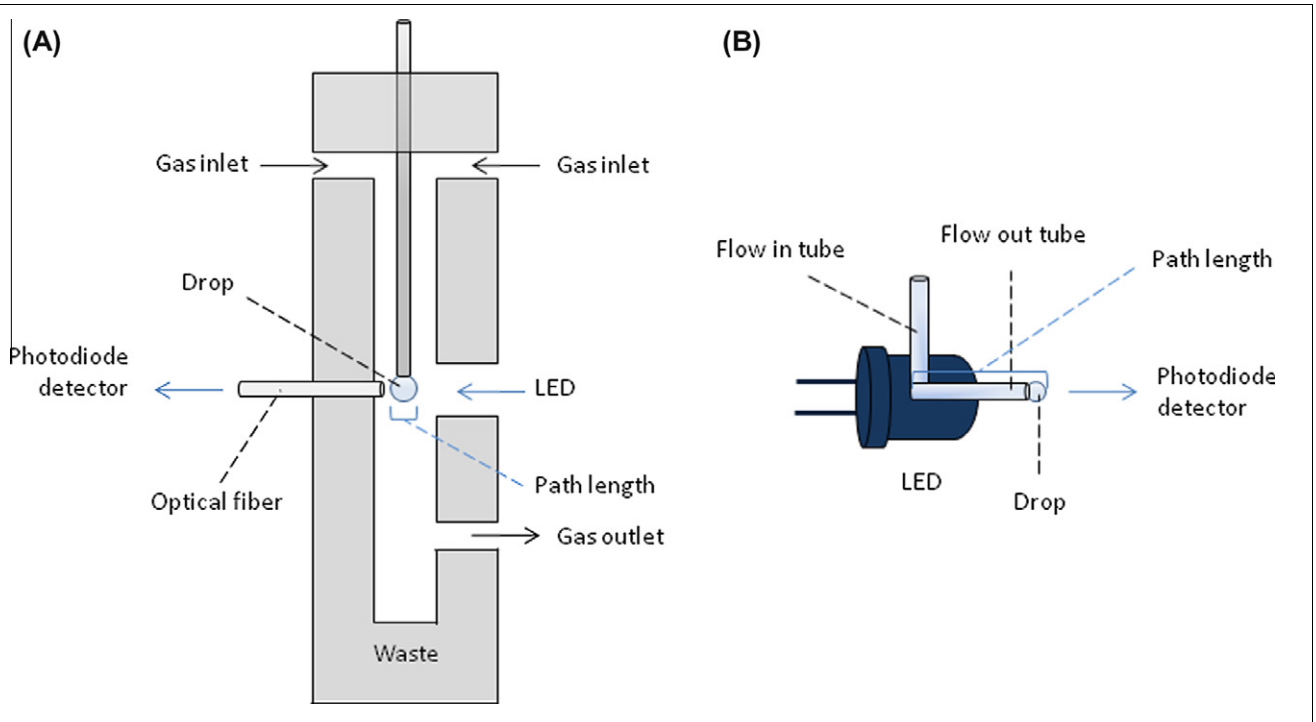

Figure 2. Drop-based systems. (A) Drop collector for gaseous analytes; and, (B) LED-based flow-through system.

levitation with UV-Vis spectrometry was demonstrated through the development of liquid-liquid extractions, acid-based microtitrations, or monitoring volatile uptake by the levitated microdrop [42,43]. In such systems, the configuration employed to achieve UV-Vis spectrometric measurements is similar to that explained above for drops or films. Recent reviews concerning the principles and the general application of levitated droplets in analytical chemistry can be consulted for further details [44,45].

3.7. Micro-total analysis systems

The search for miniaturized analytical instruments capable of analyzing nL-sized sample volumes with a high level of automation has led to the development of micro-total analysis systems (μ-TASs) [46]. The improvement in sensitivity of μ-TASs with optical detection is challenging, due to the limited optical path length commonly displayed by these systems. Some of these microfluidic systems make use of the technology described above (e.g., LCWs or TIR) to enhance the low sensitivity provided by microchips with optical detection, which results from the short path lengths employed. LCWs are being used in microfluidics to achieve the highest sensitivity possible with the nL volumes commonly used in such microfabricated microanalysis systems [47,48]. In this context, microfabrication of Teflon AF-coated LCW channels in silicon was reported by Datta et al. [49].

An optical fiber-based UV-Vis spectrometric microdetector suitable for microvolume samples (0.9 μL) was proposed by Bargiel et al. [50]. This system provides a

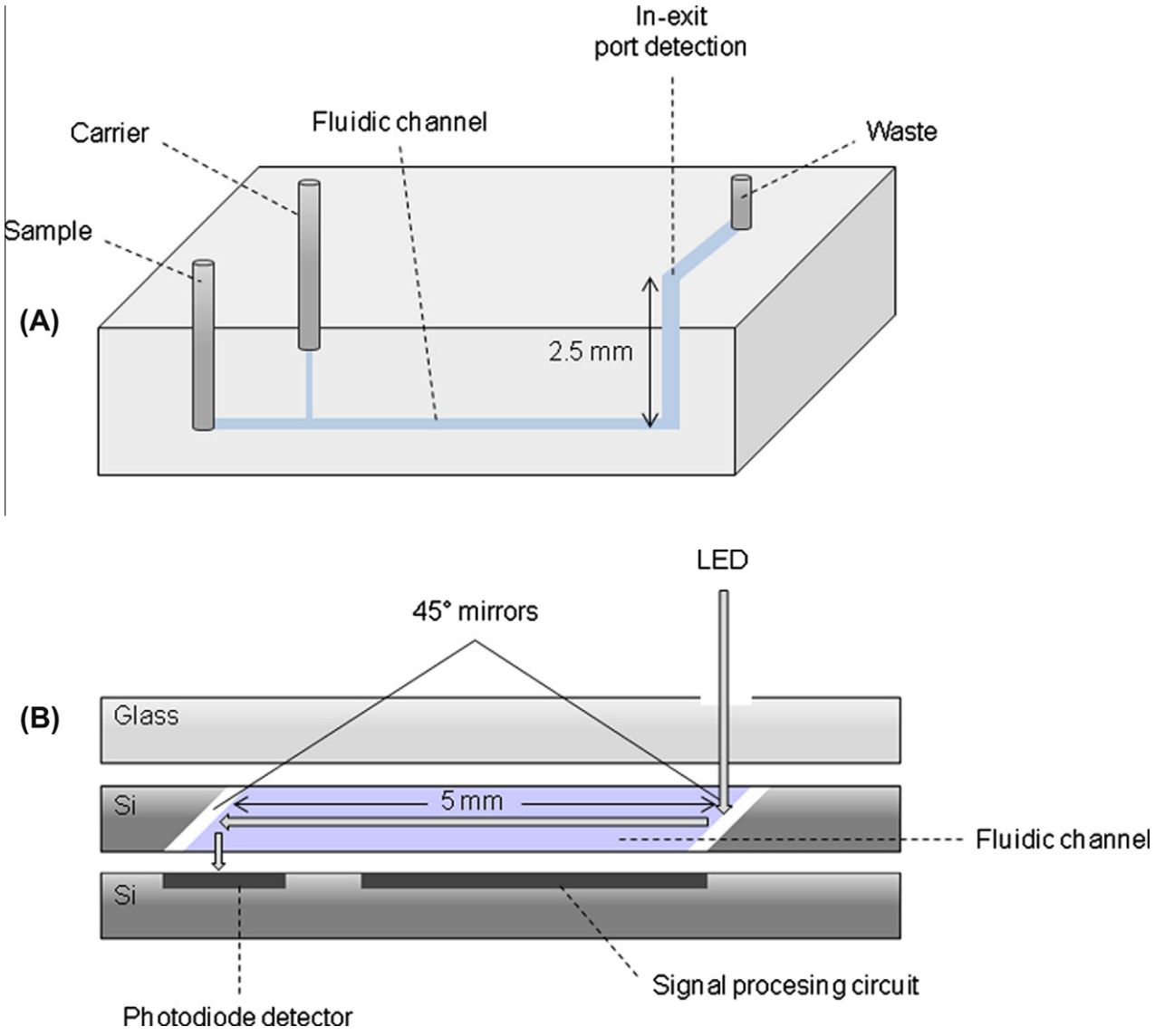

Figure 3. Microfluidic systems. (A) Detection in the exit port of disposable PDMS chips; and, (B) absorption photometry silicon microchip with improved path length by reflection in the fluidic channel.

path length of 10 mm, and is compatible with the μ-TAS technology, but also with conventional analytical instrumentation as an alternative optical cuvette.

Gáspár et al. combined a projection microscope and a fiber-optic, miniaturized UV-Vis spectrometer to perform absorbance measurements on disposable polydimethylsiloxane (PDMS) chips [51]. This instrument selects a very small area of the channel pattern in order to perform a measurement, thus allowing analysis of microvolume samples (the volume of the cell is only 120 nL) with a 2.5-mm optical path length in the chip (Fig. 3A**)**.

Minas et al. developed a portable, low-cost, disposable, lab-on-a-chip system, which combines a microfluidic system, highly selective optical filters and a detection and readout system, and is useful for UV-Vis spectrometric determination of biomolecules in biological fluids [52].

Pan et al. presented an small ($12 \times 4.5 \times 2.1$ cm), light (less than 70 g), hand-held photometer, which integrated an LCW flow cell, a light source, a photodiode detector, a dropper pump for aspiration of the sample into the flow cell, an electronic circuit for instrument control and data processing, a liquid-crystal display screen and a battery [53]. This miniaturized system determined target analytes with lower sample consumption (0.35 μL) and larger path length (15 mm) than those of conventional UV-Vis spectrometers.

Duggan et al. developed an on-chip UV-Vis spectrometric system by coupling the light source, the microfluidic chip and the detection system to form a polymer based-LCW along a single microfluidic channel [54]. The authors used different cladding materials [i.e. Teflon polytetrafluoroethylene (PTFE), Teflon fluoroethylene-propylene (FEP) and Teflon AF polymers] for the LCW. The combination of LCW with 3D-chip architecture, where the LCW was perpendicular to the mixing channel, provided a path length of 5 mm using sample volumes in the range 0.35–1.1 μL.

An absorption photometry silicon microchip was presented by Noda et al. [55]. The system, constructed in accordance with micro-electro-mechanical system (MEMS) technology, provides an extended optical path length (5 mm) as a result of using two 45° mirrors placed at each end of the fluidic channel, as shown in Fig. 3B. Accordingly, light from a LED source is irradiated from the top side and reflected by the first mirror that propagates the light through the fluidic channel. At the end of the fluidic channel the light is reflected by a second mirror, thus reaching a photodiode fabricated on the bottom layer.

TIR, also known as attenuated total reflection (ATR), was used by Steigert et al. to improve sensitivity by one order of magnitude with a centrifugal 'lab-on-a-disk' system [56]. These so-called centrifugal microfluidics platforms integrate several analytical steps by taking advantage from both centrifugal and capillary forces.

Fang et al. developed miniaturized systems based on stopped-flow liquid-liquid extraction and a Teflon AF capillary LCW [48,57]. These systems extract analytes of interest with consumption of sample and organic solvent below 10 μL and 1 μL, respectively.

3.8. Cavity ring-down absorption spectroscopy

In 1998, O'Keefe and Deacon described cavity ring-down spectroscopy (CRDS) [58], in which a pulsed laser source is coupled to an optical cavity formed by two highly reflective mirrors. The light that escapes from the cavity

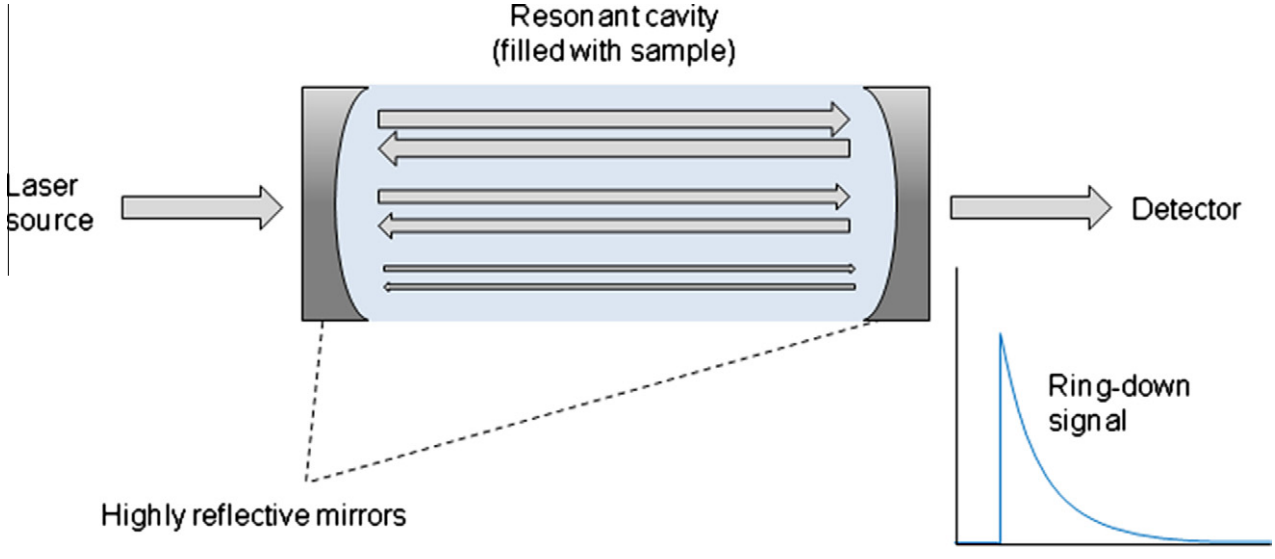

Figure 4. Cavity ring-down spectroscopy (CRDS) system.

Table 2. Selected applications of miniaturized UV-Vis spectrometric systems

Analyte(s)	Sample(s)	Measurement volume (μL)	Path length (mm)	LOD	Working range	RSD (%)	Ref.
Application of drops, films and porous tubes to atmospheric analysis							
Ammonia	Gas samples	~2.25	~2	–	up to 250 ppbv	–	[32]
Chlorine	Gas samples	18	–	–	up to 1100 ppbv	1.2	[35]
Nitrogen dioxide	Gas samples	14–57	~1	<10 ppbv	30–100 ppbv	0.24–4.71	[36]
Ozone	Gas samples	14	50	1.2 ppbv (1 min)	up to 300 ppbv (1 min)	–	[41]
				0.24 ppbv (5 min)	up to 100 ppbv (5 min)		
Application of microfluidic systems							
Iron(II)	Water samples	0.04	17	0.1 μmol/L	1–100 μmol/L	0.6	[47]
Dopamine	Pharmaceuticals	8	–	400 μg/L	1200–50000 μg/L	40–45	[68]
DNA	DNA samples	0.1	15	100 μg/L	1000–20000 μg/L	2.3	[53]
Cholesterol	Human serum			–	0.26–2 mmol/L	1.2	
Ethanol	Human whole blood	0.5	10	40 μg/L	40–2500000 μg/L	4.0	[56]
Combination of miniaturized sample preparation approaches with microvolume UV-Vis spectrometric systems							
Trimethylamine-nitrogen	Fish samples	2	1	0.006 μg/g	0.0125–0.075 μg/g	5	[69]
Formaldehyde	Cosmetics	2	1	0.02 μg/g	–	5.9	[70]
Thiols	Pharmaceuticals	2	1	74 nmol/L	500–20000 nmol/L	4.5	[71]
Chlorine	Chlorine mixtures			0.6 μg/L	7–1800 μg/L	1.1	
Ammonia	Waters			46 μg/L	200–4000 μg/L	3.3	
Iodine	Water, food and pharmaceutical samples			10 μg/L	25–750 μg/L	4.4	
Mercury	Water samples	25	–	0.2 μg/L	2–50 μg/L	4.9	[72]

is then detected by a photomultiplier. Fig. 4 shows the experimental set-up of a CRDS system.

In CRDS, the light present in the cavity decays exponentially at each reflection (mainly due to transmission/absorption by the mirrors or absorption by gases present in the cavity), giving rise to a characteristic ring-down time. When absorbing species are present inside the cavity medium, additional light is lost due to absorption, then yielding exponential light-intensity decay with shorter ring-down time [59,60]. The multiple (hundreds or thousands) reflections that the radiation pulse undergoes in the optical cavity greatly increase effective path length in such systems, so enhancing sensitivity. Furthermore, this technique is characterized for being insensitive to light-source-intensity fluctuations.

Even though most applications have been for analysis of gas samples, substantial efforts have been devoted to applying CRDS to the analysis of liquid phases [61]. The development of CRDS systems for analysis of very small amounts of sample is a challenge towards their miniaturization and compatibility with analytical microdevices. In recent years, several researchers have developed ring-down absorption spectroscopy systems capable of analyzing liquid-phase volumes as small as a few nL while providing outstanding sensitivity as a result of the multipass nature of these techniques [62–65]. The features of this promising technique are discussed in detail in several excellent review articles [59,60,66,67].

4. Selected applications

The microvolume systems described above enable UV-Vis spectrometric analysis with negligible sample consumption. Different commercial microvolume UV-Vis spectrometric systems are increasingly being used in routine laboratories devoted to life sciences as a result of their advantages (e.g., negligible sample consumption, rapidity, portability, avoidance of sample dilution and cuvette cleaning) compared with conventional UV-Vis spectrometry.

As for analytical chemistry, the use of such systems seems to be at its early stages, probably because the first systems developed for microsample quantitation were clearly promoted by life scientists, who obviously have different needs and requirements. Apart from the direct use of microvolume UV-Vis spectrometric systems described above, analytical chemists used different strategies in order to exploit applicability at trace levels. Table 2 shows selected applications of microvolume UV-Vis spectrometric systems to the analysis of environmental, food, clinical, cosmetic and pharmaceutical samples.

4.1. Application of drops, films and porous tubes

Since its inception in 1995, several applications have been developed employing drops and/or films as the sample compartment for direct UV-Vis spectrometric

measurement. Specifically, the development of different analytical approaches for atmospheric analysis was tackled by Dasgupta's group using drops and liquid films as not only sample compartment but also extractant phase of the corresponding volatile analyte. Thus, Cardoso and Dasgupta employed a liquid film of Griess/Saltzman reagent as extractant phase of nitrogen dioxide, achieving a limit of detection (LOD) lower than 10 ppb by volume using a 5-min sampling time [36].

Also, a drop-based sensor was proposed for determination of gaseous chorine. The method is based on the collection of the volatile analyte onto an 18-μL drop containing tetramethylbenzidine, and subsequent reaction yielding a yellow product that can be determined by UV-Vis spectrometry [35]. Fig. 2A shows the systems employed for the analysis of gaseous samples.

Dasgupta and co-workers also demonstrated the analytical usefulness of porous tubes as collectors of volatile analytes [41]. In such systems, porous tubes are filled with a selective reagent-containing aqueous solution and exposed to a continuous flow of gaseous sample. Low LODs were obtained with these systems for nitrogen dioxide and ozone, on the basis of the Griess-Saltzman and indigotrisulfonate colorimetric assays, respectively.

4.2. Application of microfluidic systems

Different microfluidic systems with UV-Vis spectrometric detection were developed and employed for the analysis of microvolumes of samples in a wide variety of matrices.

A microfluidic flow-injection-analysis system with an LCW flow cell was presented by Du et al. [47] for high-throughput UV-Vis spectrometric determination of Fe(II) by complexation with o-phenantroline. The system provides better sensitivity than conventional UV-Vis spectrometry, as a result of an effective path length of 17 mm, with a cell volume of 40 nL.

Maminski et al. proposed two UV-Vis spectrometric procedures for the determination of dopamine in pharmaceutical samples, employing a microfluidic reaction chamber based on PDMS technology [68]. An 8-μL flow-through cuvette was used in combination with a 6-μL Y-shaped microreactor for absorbance measurement. The procedures developed involved coupling dopamine with isoniazid and reaction with sodium nitroprusside, respectively.

Pan et al. applied a portable, miniaturized photometer based on LCW absorption detection to the analysis of DNA samples and determination of cholesterol in human-serum samples, respectively [53]. The use of dual-wavelength detection (by employing two UV LEDs (λ = 260 nm and 280 nm) as light sources) allowed assessment of the quality of DNA, while a single, bluish-green LED source (λ = 497 nm) was used for the determination of total cholesterol in clinical samples.

A colorimetric assay for the determination of ethanol in a single drop (0.5 μL) of untreated human whole blood was reported by Steigert et al., using a centrifugal ''lab-on-a-disk'' system [56]. The assay protocol is based on four different steps. In the first, a 0.5-μL sample of blood is metered precisely and the disk is spun at 35 Hz to transport the liquids to the detection cell. The direction of rotation is frequently reversed to improve mixing at a lower frequency (~10 Hz) and, subsequently, the frequency is increased to 30 Hz to clear the cellular components of the blood. Finally, ethanol is determined by a two-step enzymatic reaction with spinning at 8 Hz.

4.3. Combination of miniaturized approaches to sample preparation with microvolume UV-Vis spectrometric systems

Several UV-Vis spectrometric-based analytical methods involve use of large amounts of reagents, including toxic organic solvents. Efforts to develop miniaturized microvolume systems commented on above are also reflected in the improvement of these analytical methods, since available miniaturized sample-preparation techniques can be combined with them, resulting in large decreases in the reagents needed and the waste produced. In this way, the combination of microvolume UV-Vis spectrometry with miniaturized approaches to sample preparation [e.g., liquid-phase microextraction (LPME)] constitutes an appropriate way to improve conventional analytical methods in terms of sensitivity (due to the large enrichment factors provided by these techniques), integration of steps and consumption of reagents.

A few examples concerning the coupling of microvolume UV-Vis spectrometry with LPME approaches can be found in the literature. For example, improvements and miniaturization of the AOAC Official Method 971.14 for determination of trimethylamine-nitrogen (TMA-N) in fish samples [69], and the European Official Method of Analysis of formaldehyde in cosmetic products [70] were achieved using confined based-drop systems in combination with headspace single-drop microextraction (HS-SDME) and ultrasound-assisted emulsification microextraction, respectively. In both cases, low LODs were obtained as a result of the large enrichment factors achieved with LPME, which largely compensated for the reduced path length provided by the microvolume UV-Vis spectrometer employed.

Sharma et al. employed the same confined drop-based system in combination with different modes of LPME (direct-SDME, HS-SDME and liquid-liquid-liquid microextraction) and solid-phase extraction (SPE) for the determination of thiols, chlorine, ammonia and iodine in a variety of sample matrices [71].

Yang et al. presented a direct-SDME method, in which an organic drop was used as collector and sample compartment for the determination of mercury in water samples, and an LED and a hand-held charge coupled device (CCD) as radiation source and detector,

respectively [72]. The method, based on extraction and in-drop derivatization of mercury by a 25-μL microdrop, comprising carbon tetrachloride and dithizone, provided an enrichment factor of 69 after 15 min microextraction.

5. Concluding remarks

Miniaturization of detection systems is a challenge that has been met with different degrees of success in analytical chemistry. Specifically, miniaturization of conventional UV-Vis spectrometry has been achieved to some extent by implementing different technological advances. Thus, several miniaturized UV-Vis spectrometers and/or accessories compatible with conventional instrumentation are currently available, allowing the UV-Vis spectrometric determination of target analytes with negligible sample consumption and increased path length-to-detection volume ratio as compared to classical UV-Vis spectrometers. Nonetheless, additional work is needed to improve the performance of such microvolume systems, thereby meeting current needs in analytical chemistry, especially with respect to sensitivity and simplicity.

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